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Novel Method For Tyrosine Assessment in vitro by Using Luminescence Quenching of The Nano Optical Sensor Eu-Ciprofloxacin Complex Doped in Sol-Gel Matrix

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Abstract

A low cost and very sensitive method for the assessment of the tyrosine in blood serum, urine and hair samples was developed. The method was based upon measuring the luminescence intensity of the nano optical sensor Eu-Ciprofloxacin complex doped in sol-gel matrix at 617 nm and in presence of different concentrations of tyrosine in acetonitrile at (pH 9.1, λ_{ex}=380 nm). The significant quenching of the luminescence intensity of Eu-Ciprofloxacin complex by different concentrations of tyrosine was successfully used as an optical sensor in acetonitrile at 617 nm. The effect of different analytical parameters, e.g. pH, solvent, tyrosine concentration and foreign ions concentrations were fully accomplished. The calibration plot was achieved over the tyrosine concentration range 1.2×10^{-6} – 1.0×10^{-9} mol L^{-1}, with a correlation coefficient of 0.997 and a detection limit of 1.0×10^{-10} mol L^{-1}. The developed method is simple and proceeds without practical artifacts compared to other published methods.

Keywords: Tyrosine; Eu-Ciprofloxacin complex; Nano optical sensor; Luminescence intensity; Quenching.

Introduction

Tyrosine is a nonessential amino acid that synthesized from phenylalanine in the body. It is responsible for the conformation of almost all proteins in the human body and functions as the precursor of several neurotransmitters, including L-dopa, dopamine, norepinephrine, and epinephrine [1, 2]. Parkinson's disease, depression, and other mood disorders are generally found when tyrosine levels are abnormal [3]. In addition, tyrosine and other amino acids may play roles in inducing dementia, e.g., Alzheimer's disease [4]. For this importance of tyrosine in biological systems, a number of methods have been developed to determine its concentration in vivo, mainly using separation techniques such as high-performance liquid chromatography [5–9]. Further improvements in sensitivity are possible when mass spectrometry is combined with these separation techniques [10–12]. However, these methods suffer from low detection speeds and/or high expenses. Alternative methods involving enzymes have been proposed to generate spectroscopic [13, 14] or electrochemical [15] signals from tyrosinase. These methods provide sufficient sensitivity and selectivity but suffer from any degradation in the enzyme's activity. Fluorimetric [16] and chemiluminescence [17] methods have also been proposed for the detection of tyrosine, but these methods typically require pre-cleaning of the matrix and pretreatment of the sample. The aim of the present work is to develop a novel method for the assessment of tyrosine concentration in serum, urine and hair samples by using a nano sensor of a very good analytical performance in terms of sensitivity, time of response, stability, and reproducibility. The assay is based on the quenching of the characteristic emission bands of Eu-Ciprofloxacin nanooptode thin film that doped in silica-gel matrix.

2. Experimental

2.1. Chemicals and Reagents

All chemicals used in this work are of analytical grade and used without further purification. Ciprofloxacine (99.9 %), creatinine, glucose, uric acid, urea, and benzoic acid were purchased from Sigma. The europium oxide Eu_2O_3 (99.9%) was delivered from Chem Pur GmbH). Amino acids; tyrosine, glycine, histidine, phenylalanine, proline, serine and tryptophan were purchased from Sigma. Metal salts; $CaCl_2$, $MgCl_2$, $SrCl_3$, $AlCl_3$, NaCl, KCl, $ZnCl_2$, $CuCl_2$, $MnCl_2$, $FeCl_2$, $AgNO_3$, $HgCl_2$, $AsCl_3$, $PbCl_2$ and $SbCl_3$, were purchased from Sigma. Diethoxydimethylsilane (DEDMS) and tetraethoxysilane (TEOS) were purchased from Sigma-Aldrich. Different colored hair samples were obtained from different volunteers. Double distilled water was used in stock preparations and dilutions.

The europium nitrate salt, $Eu(NO_3)_3.6H_2O$ was prepared by addition of HNO_3 to the oxide and evaporation to dryness. Stock solutions (1.0×10^{-2} mol L^{-1}) of tyrosine, ciprofloxacin and Eu^{3+} ion were prepared by dissolving the appropriate amounts in ethanol. The standard solutions of tyrosine (1.0×10^{-4}–1.0×10^{-9} mol L^{-1}), ciprofloxacin (2.0×10^{-3} mol L^{-1}) and Eu^{3+} ion (1.0×10^{-3} mol L^{-1}) were freshly prepared by dilution in acetonitrile. NH_4OH/NH_4Cl buffer solution (pH 9.1) was prepared by mixing of 143.3 mL NH_4OH (27 %) with 75.65 g of NH_4Cl in 1.0 L distilled water. Stock and working standard solutions were stored at 0–4 ˚C. The luminescence intensity was measured at $\lambda_{ex}/\ \lambda_{em}$ = 380/617 nm.

2.2. Synthesis of Eu-Ciprofloxacine complex

The Eu-Ciprofloxacine complex was prepared [18, 19] by mixing 1.0×10^{-2} mol L^{-1} of $Eu(NO_3)_3.6H_2O$ (0.218 g) with 2.0×10^{-2} mol L^{-1} of ciprofloxacin

(0.331 g) in 50 mL ethanol. The mixture was stirred at room temperature for 15 minutes, heated at 70 ºC for 2 hr and left to cool at room temperature. Then, the precipitated product was filtered and washed with ethanol. The white-pink complex was purified by recrystallization from ethyl acetate. Fig. 1 describes the structure of Eu-Ciprofloxacin complex.

2.3. Fabrication of nano optical sensor of Eu-Ciprofloxacine complex doped in sol-gel matrix

Nano sensors prepared previously [20-22] by sol-gel method were suffer from some disadvantages in which the cracking was taken place for the product and the casted optical sensor, which is degraded into small species. Some modification was introduced to the method in which the sol after reflux was transferred into deep freezer at zero temperature to expel the trapped gases and to decrease the cracking process of the optical sensor. Even though the cracking still takes place. To overcome this problem, the preparative method was modified by adding a plasticizer (DEDMS) at room temperature. In brief, 15 mg of Eu-Ciprofloxacin complex (1.0×10^{-3} mol L^{-1}) was dissolved in 10.0 mL ethanol. 8.0 ml of the ethanolic solution was mixed with 4.0 mL of TMOS, 4.0 mL of DEDMS and 2.0 mL of water. 9.0 mL of the solution mixture was kept in a glass vial (diameter 24 mm, height 48 mm). After 6 days, a thin film was prepared from the partly hydrolyzed and condensed solution by spin-coating on a small quartz slide (width 8.5 mm; height 25 mm) to suit the cuvette of fluorimeter. The quartz slide (substrate) was cleaned by rinsing in a diluted surfactant and sonication for 30 min. Secondly, the substrate was cleaned from the surfactant residues by ultrasonication in distilled water for 30 min, followed by sonication for another 10 min in acetone. Before spin-coating, the substrate was boiled for

10 min in 2-propanol and finally spun dry. Thereafter, the partially hydrolyzed and condensed sol-gel solution was dropped [23] on the substrate with a syringe throughout a 2.0 cm filter and spin-coated at 2000 rpm for 30 s, and the TEM image of nano optical sensor is shown in Fig. 2.

2.3. Instrumentations

All luminescence measurements were recorded with a Meslo-PN (222-263000) Thermo Scientific Lumina fluorescence spectrometer in the range (190 – 900 nm). The absorption spectrum of the samples was recorded in the range of 220–750 nm with Thremo Scientific UV-Visible spectrophotometer. A calibrated pHs-JAN-WAY 3040 ion analyzer was used to adjust the pH of all measured solutions. The separation of protein from samples was carried out by centrifuging of samples for 15 min and 4000 rpm. The morphology of Eu-Ciprofloxacin thin film nano optical sensor was performed by JEOL JEM-1230 at 120 Kv.

2.4. Analytical applications

The analytical determinations of tyrosine in serum, urine and hair samples were performed by measuring the luminescence intensity of the Eu-Ciprofloxacin nano optical sensor before and after addition of tyrosine to the prepared test sample solutions. The change in luminescence intensity was utilized for the determination of tyrosine in the test samples.

2.4.1. Preparation of the test solutions of serum samples

3.0 mL of citrate solution was added to 4.0 mL plasma and the solution was centrifuged for 15.0 min at 4000 rpm to remove all proteins. After decantation, 1.0 mL of the serum was added with 0.1 mL of the buffer (pH=9.1) to the thin film nano of the optical sensor in the cuvette, and finally the 1.9 mL of acetonitrile was added to give the test solution.

2.4.2. Preparation of the test solutions of urine samples

Urine samples was carefully collected from healthy volunteers (neither medicines nor vegetarian diets were applied) over a 24 h period and stored at 4 ◦C. 10.0 mL of acetonitrile were added to 10.0 mL of a urine sample and the solution mixture was kept in an ice bath for 30 min. After centrifugation for 10 min at 4000 rpm and 4 ◦C, a precipitate was obtained. After decantation, the supernatant solution was evaporated at 100 ◦C until dryness. The residue was dissolved with 1.0 mL acetonitrile to give the test sample solution [24].

2.4.3. Preparation of the test solutions of hair samples

10.0 mg of washed, dried hair sample was dissolved in 1.0 mL of 1.0 mol L^{-1} NaOH in the presence of Na_2SO_4 and the reaction mixture was heated to 70–90°C for 30 min. The test sample solution [25] was attained by dilution of the product solution to 25.0 mL by acetonitrile and adjustment of pH to 9.1.

3. Results and discussion

3.1 Spectral characteristics

The UV–vis absorption spectra of tyrosine (1.0×10^{-4} mol L^{-1}), Eu-Ciprofloxacine nano optical sensor in sol gel matrix and in the presence of tyrosine (1.0×10^{-4} mol L^{-1}) are shown in Fig. 3. The spectrum of tyrosine (1) shows an intense broad absorption band at (276 nm) while the spectrum of optical sensor (2) shows two broad bands in the UV region (278 and 322 nm). Upon addition of the tyrosine to optical sensor in acetonitrile, the intensity of 278 nm band increased due to the aromatic ring of tyrosine and the intensity of 322 nm band decreased. This behavior reveals that tyrosine quenched the energy of the optical sensor in the ground state. At pH 9.1 and in acetonitrile, the emission spectrum of the optical sensor after excitation at

380 nm gives the characteristics emission bands of Eu^{3+} ($^5D_0 \rightarrow {}^7F_1$, $^5D_0 \rightarrow {}^7F_2$, $^5D_0 \rightarrow {}^7F_3$, $^5D_0 \rightarrow {}^7F_4$, and $^5D_0 \rightarrow {}^7F_5$). This is due to the effective energy transfer from triplet state of the ciprofloxacin to the excited energy state of Eu^{3+} ($^5D_0$) that followed by relaxation from the $^5D_0$ to different ground energy states of Eu^{3+}. Upon addition of 1×10^{-4} mol L^{-1} tyrosine to the nano optical sensor in sol gel matrix, the intensity of all emission bands of Eu^{3+} decreases, specially the electrical band at 617 nm as a result of quenching of the energy of the excited state of Eu^{3+} ($^5D_0$) by tyrosine, Fig. 4. However, tyrosine contains free OH oscillator, is negatively charged at pH=9.1, capable of forming an ion-pair with the positively charged nano optical sensor and in turns the emission intensity of all bands are decreased after the addition of different concentrations of tyrosine (1.0×10^{-4}-1.0×10^{-9} mol L^{-1}). Accordingly, Eu-Ciprofloxacin is proficient optical sensor for tyrosine in acetonitrile at pH 9.1, Fig. 5.

3.2 Effect of Solvent

The influences of the solvent on the fluorescence intensity of thin film Eu-Ciprofloxacin doped in sol–gel matrix under the conditions established above are shown in Fig. 6. The results show that higher luminescence intensity of the nano optical sensor in aprotic solvents (acetonitrile and DMF) was mainly attributed to the formation of anhydrous solvates of the binary ion-pair associates. While, the lower luminescence intensity that observed for protic solvents (water and ethanol), was explained in terms of efficient quenching of the excited state of the nano optical sensor by interactions with high-energy vibration oscillators, like -OH groups [26-29]. Therefore, all the experiments are carried out in the presence of acetonitrile.

3.3 Effect of pH

pH of the solution is one of the principal factors that influencing the ionization of functional groups and in turns controlling the surface charge of the species. Tyrosine contains several functional groups that ionize at different pH values. Four distinct tyrosine species are possible—namely R^+, R, R^-, and R^{--} based on their total charge. At pH 5.6, the tyrosine exists in R form and has a band at 276 nm and at pH > 9.0; tyrosine appears only in forms that possess negative charges. Actually, at pH 9.1 and 10.5, the predominant species are R^- and R^{--}, respectively. The position of the absorption band at 276 nm that observed in acidic medium, was slightly shifted at higher pH to 290 and 316 nm. This is due to the deprotonation of acidic and phenolic groups of tyrosine at pH 9.1 and 10.5, respectively [2]. Based on the p*K* values of the deprotonation and protonation of the carboxylic and amino groups, respectively. At pH 9.1, tyrosine will be in negatively charged (R^- form); it will attack the axial coordination sites of the Eu-Ciprofloxacin complex in the nano optical sensor leaving other uncharged species in solution.

3.4 Interferences in the detection of tyrosine by the proposed method

The effects of potentially interfering agents on the determination of tyrosine in different biological samples by the proposed method were investigated. An equimolar concentration of 6 amino acids was individually added to tyrosine in bovine serum and hair samples. The concentration of tyrosine was maintained at 1.0×10^{-4} mol L^{-1} and pH was adjusted to 9.1 for all samples. At this pH, the mono-negatively charged tyrosine (R^- form) will attack the Eu-Ciprofloxacin complex in the nano optical sensor leaving other interfering amino acids, neutral in solution. Fig. 7 shows the percentage interfering ratio (% ΔF) at 617 nm band for the different amino acids. In

addition, the effect of metal salts ($1.0x10^{-4}$ mol L^{-1}) in hair samples was examined, and % ΔF at 617 nm band was calculated as shown in Fig. 8. The results are direct proof that the assessment of tyrosine by the proposed method in biological and hair samples is feasible.

3.5 *Calibration curve and detection limits*

The effect of tyrosine concentration on luminescence intensity of the nano optical sensor according to Stern-Völmer equation [30], was presented in Fig. 9. The luminescence intensity at 617 nm is linearly decreased with the concentrations of tyrosine over the range 1.0×10^{-9} to 1.2×10^{-6} mol L^{-1} with a correlation coefficient R^2 of 0.997. However, the linear form of Stern-Völmer equation is given by Eq.1;

$$(F_o/F) - 1 = K_{sv}\,[Q]\,n \qquad \text{Eq.1}$$

where F_o and F are the luminescence intensities of the nano optical sensor in absence and presence of the tyrosine, respectively, [Q] is the concentration of the tyrosine, and K_{sv} is called Stern-Völmer constant, equals to.

The value of the Stern-Völmer constant K_{sv} was determined from the slope of the plot of the luminescence intensities against the concentration of the tyrosine, and was found to be 0.0032 L mol $^{-1}$. Accordingly, the critical concentration C_O and the critical radius R_o (defines as the distance between tyrosine and the optical sensor) are calculated from Eqs 2 and 3, as follow:

$$C_o = \frac{1}{K_{sv}} \qquad \text{Eq. 2}$$

$$R_o = 7.35 C_O^{-1/3} \qquad \text{Eq. 3}$$

The values of C_o was found to be 312 mol L^{-1} and R_o = 1.96 Å. The critical radius R_o <10 Å, indicates that the quenching mechanism is electron transfer, Fig. 10.

The detection limit (LOD) and quantification detection limit (LOQ) were calculated according to ICH guidelines [31] using the formulae: LOD = 3.3 S/b and LOQ = 10 S/b (where S is the standard deviation of blank luminescence intensity values, and b is the slope of the calibration plot). The statistical results of regression for the calibration plot are presented in Table 1.

The comparison of the proposed thin film nano optical sensor for the determination of tyrosine with other published methods was presented in Table 2. The items of comparison ensures that the developed method has good stability, lower limit of detection (1.0×10^{-10} mol L^{-1}) and wide linear range of applications ($1.0\times10^{-9} - 1.2\times10^{-6}$ mol L^{-1}).

3.6 *Accuracy and precision of the proposed method*

To compute the accuracy and precision, the described assays were repeated three times within the day to determine the repeatability (intra-day precision) and three times in different days to determine the intermediate precision (inter-day precision) of the proposed method. The results of this study are summarized in Table 3. The percentage relative standard deviation (%RSD) values were in range of 0.12-0.21% (intra-day), 0.14-0.26 % (inter-day) for serum, urine and hair samples indicating high precision of the proposed method. Accuracy was evaluated as percentage relative error (%RE) between the measured mean concentrations and the actual concentration of tyrosine. Bias {bias % = [(average actual found - actual concentration)×100/ actual concentration]} was calculated for each

concentration and these results are also presented in Table 3. The values of percent relative error (%RE) were found to be 2.38-5.00 % (intra-day) and 2.5- 6.89% (inter-day) for serum, urine and hair samples indicating high accuracy of the proposed method.

3.7 Determination of tyrosine in different physiological samples by Eu-Ciprofloxacin nano optical sensor

In order to evaluate the performance of the proposed optical sensor, the assessment of tyrosine was carried out in the *serum* of different human samples. The experimental results of the test solutions are compiled in Table 4. The average recovery and relative standard deviation are 95.0-105.0 % and 0.16-0.37 %, respectively. This ensures that that the developed method can be easily performed, offers good precision and accuracy for the determination of tyrosine in serum samples.

In addition, the proposed method was also applied for the assessment of tyrosine in urine and hair samples. The results of this study were presented in Table 4. The average recovery and relative standard deviation for the urine samples and hair samples were (97.6-105.2 % and 0.26-0.32 %) and (95.0-103.4 % and 0.31-0.41 %), respectively.

4. **Conclusion**

The present work affords a simple, sensitive and selective complex of Eu-Ciprofloxacin in sol–gel matrix as nano optical sensor for the determination of tyrosine in acetonitrile by fluorescence technique. The thin film of complex is able to detect tyrosine by the quenching of its characteristic emission band at 617 nm. The sensitivity of the nano optical sensor towards tyrosine is explained by the formation of the ion-pair associates at pH 9.1. The selectivity of the sensor was proved by tyrosine determinations in the presence of different amino acids and metal salts as interfering species. The

proposed method was tested for 1.2×10^{-6} – 1.0×10^{-9} mol L^{-1} tyrosine concentration range with a correlation coefficient of 0.997 and a detection limit of 1.0×10^{-10} mol L^{-1}. Finally, the assessment of tyrosine in different blood serum, urine and hair samples was successfully achieved by the proposed method.

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Figures:

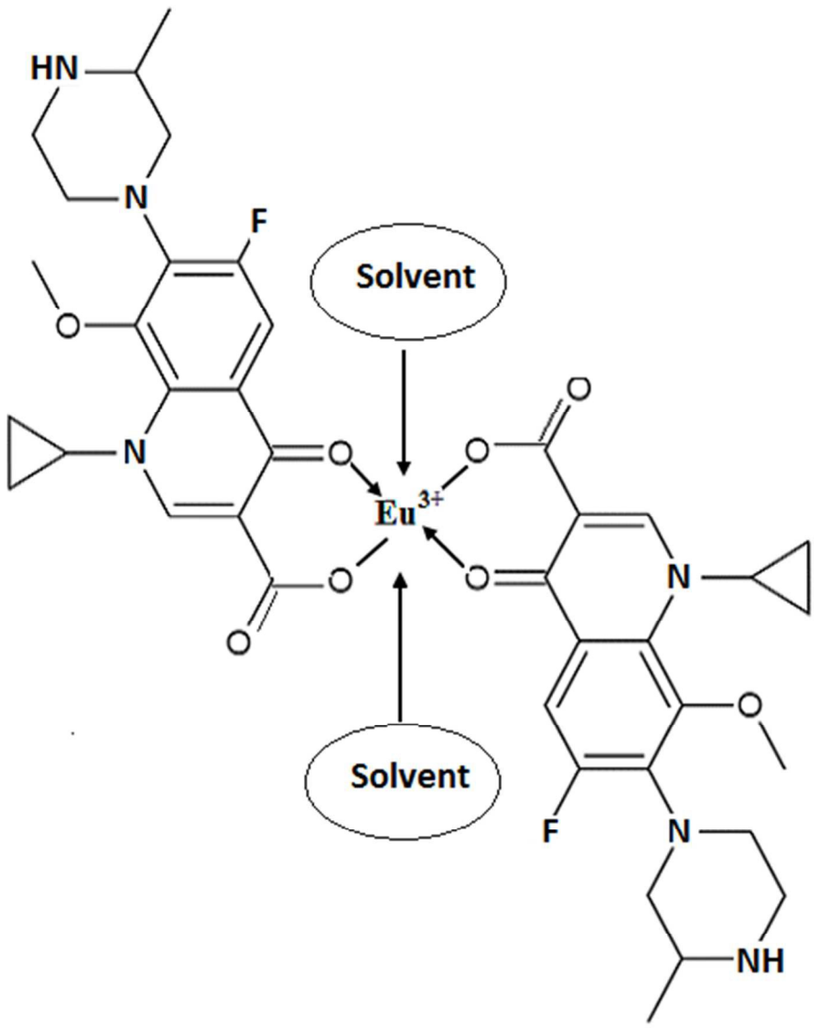

Fig. 1. Structure of Eu-Ciprofloxacin complex

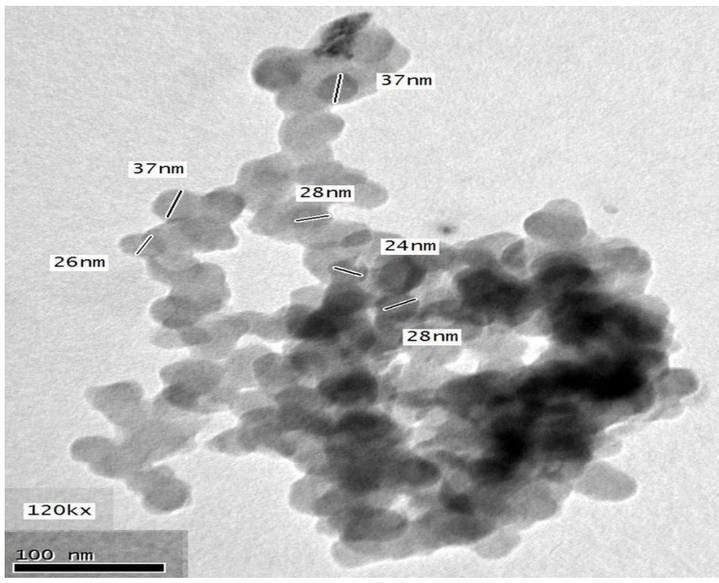

Fig. 2. TEM image of the nano optical sensor

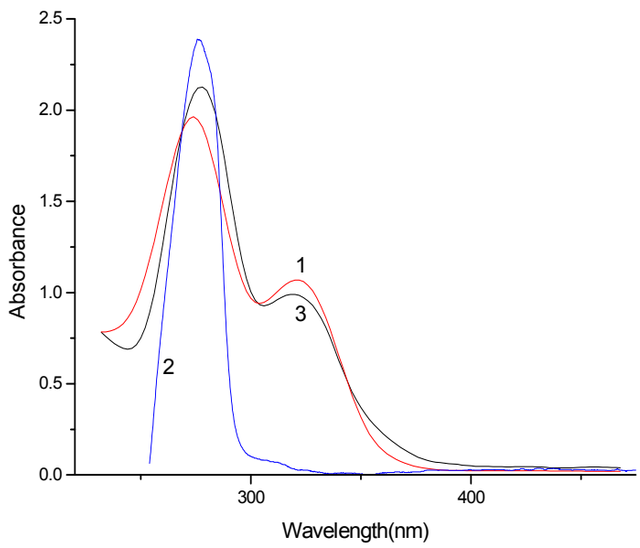

Fig. 3. Absorption spectra of (1)- nano Eu-Ciprofloxacin complex doped in sol-gel matrix, (2)-1x10^{-4} mol/L of tyrosine and (3)- nano Eu-Ciprofloxacin complex doped in sol-gel matrix with 1x10^{-4} mol/L of tyrosine.

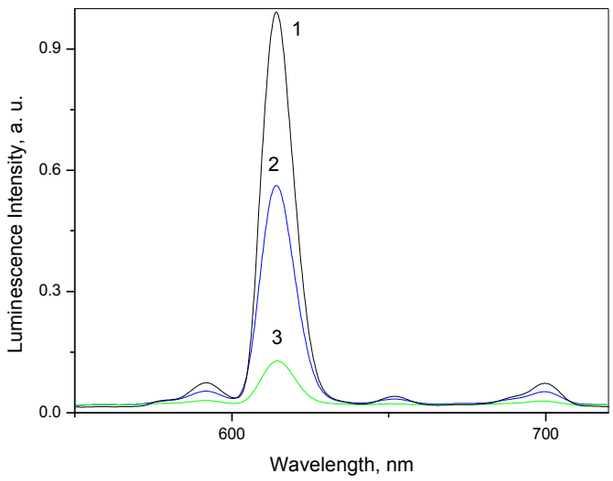

Fig. 4. Luminescence emission spectra of (1)- nano Eu-Ciprofloxacin complex doped in sol-gel matrix (2)-Eu-Ciprofloxacin in presence of $1x10^{-7}$ mol/L tyrosine and (3)-Eu-Ciprofloxacin in presence of $1x10^{-4}$ mol/L in acetonitrile, pH= 9.1 and λ_{ex}=380 nm.

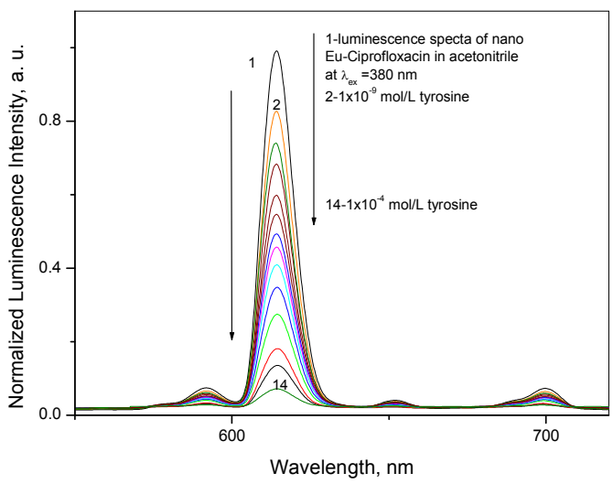

Fig. 5. Luminescence emission spectra of nano Eu-Ciprofloxacin complex doped in sol-gel matrix in the presence of different concentrations of tyrosine in acetonitrile, pH= 9.1 and λ_{ex}=380 nm.

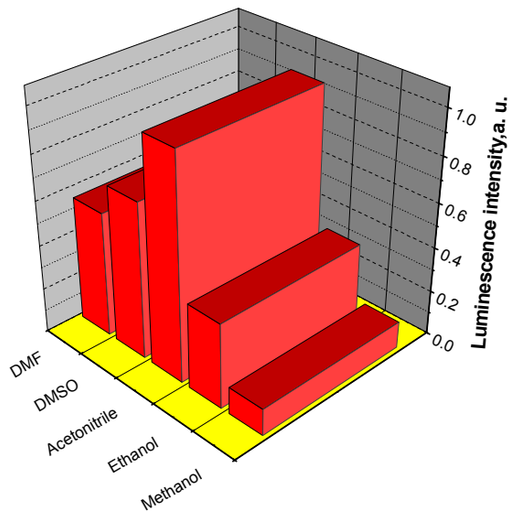

Fig. 6. The effect of solvent on the luminescence spectrum of nano Eu-Ciprofloxacin complex doped in sol-gel matrix at λ_{ex}=380 nm.

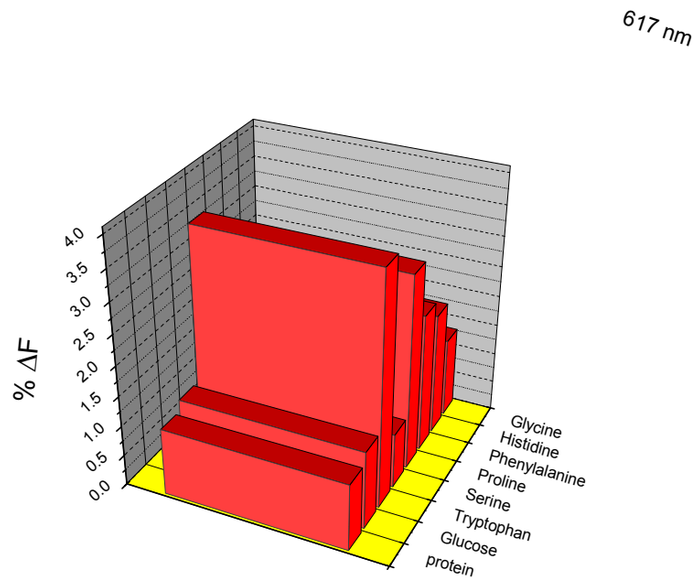

Fig. 7. The interfering percentage % ΔF against the interfering species at 617 nm band. % ΔF = [Δf (different interfering species)/ Δf (tyrosine)]x 100. Δf = the difference between the luminescence intensity of the sensor in absence and in presence of amino acids.

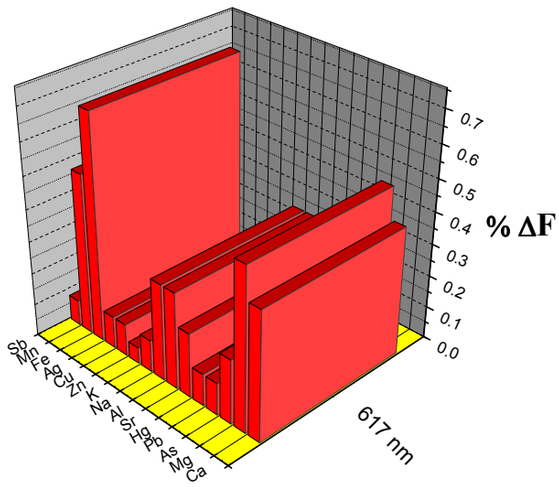

Fig. 8. The interfering percentage % ΔF against the interfering species at 617 nm band. % ΔF = [Δf (different interfering species)/ Δf (tyrosine)]x 100. Δf = the difference between the luminescence intensity of the sensor in absence and presence of metal salts.

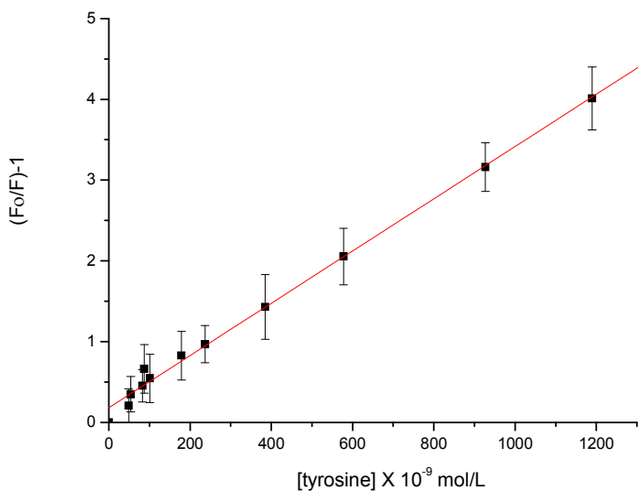

Fig. 9. Linear relationship between (F_o/F-1) against tyrosine concentrations.

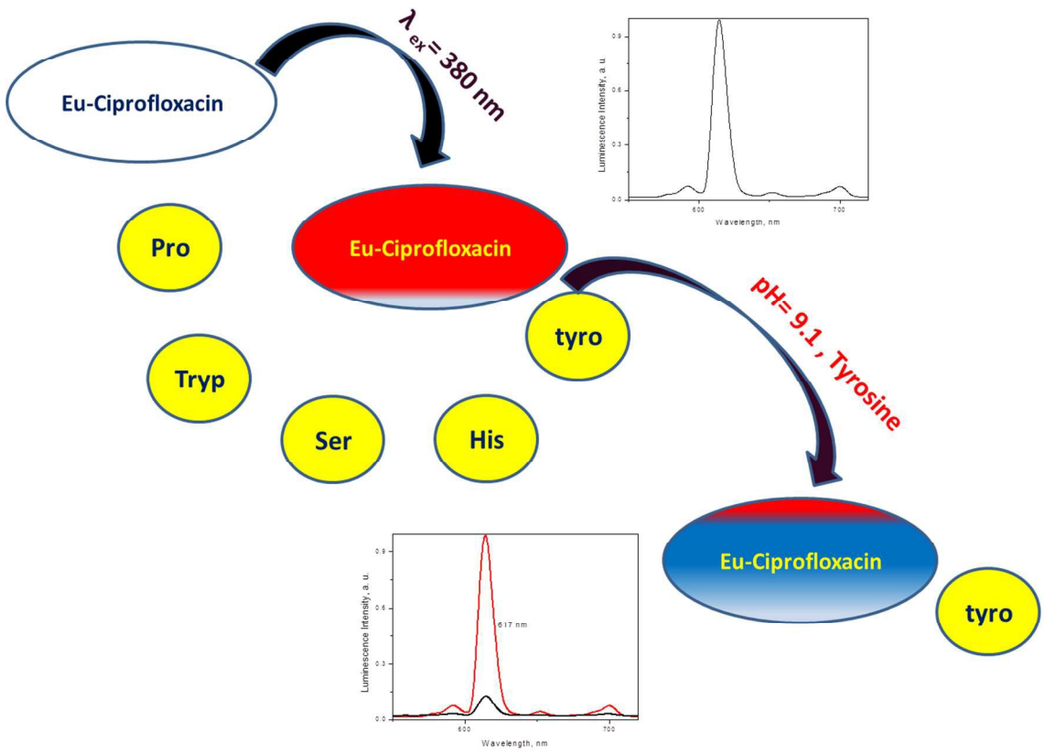

Fig.10. Mechanism of the quenching process

Tables:

Table 1. Sensitivity and regression parameters for the nano optical sensor.

Parameter	values
λ_{em}, nm	617
Linear range, mol L^{-1}	$1.2 \times 10^{-6} - 1.0 \times 10^{-9}$
Limit of detection (LOD), mol L^{-1}	1.0×10^{-10}
Limit of quantification (LOQ), mol L^{-1}	3.0×10^{-10}
Regression equation, Y*	
Intercept (*a*)	0.17
Slope (*b*)	0.003
Standard deviation	0.098
Variance (Sa^2)	9.6×10^{-3}
Regression coefficient (r)	0.997

*$Y=a+bX$, Where Y is luminescence intensity, X is concentration in mol L^{-1}, *a* is intercept, *b* is slope.

Table 2: Comparison of Nano optical Sensor method with different methods of the determination of tyrosine

Sample tyrosine	Method	Linear range (mol L^{-1})	Detection limit (mol L^{-1})	References
Compound amino acid injection	Microcolumn electrophoresis	$(0.60–90) \times 10^{-6}$	0.20×10^{-6}	[32]
Serum	UV spectrophotometer	$(0–64) \times 10^{-6}$	5×10^{-6}	[33]
Urine	HPLC–APCI–MS/MS	$(0.03–10)\times10^{-6}$	0.025×10^{-6}	[34]
Pharmaceutical	Cyclic voltammetry	$1\times10^{-7} – 5\times10^{-5}$	8×10^{-8}	[35]
Pharmaceutical and serum samples	NRD Photo Probe	$1\times10^{-5} – 5\times10^{-9}$	2.7×10^{-9}	[2]
Serum, urine and hair samples	Nano optical Sensor	$1.2\times10^{-6} – 1.0\times10^{-9}$	1.0×10^{-10}	present work

Table 3. Evaluation of intra-day and inter-day accuracy and precision**.**

Sample	Tyrosine added*	Total tyrosine found*	Actual tyrosine found *	Intra-day accuracy and precision (n=3)			Inter-day accuracy and precision (n=3)		
				Tyrosine Average (found ±CL)*	%RE*	%RSD*	Tyrosine Average (found ±CL)*	%RE*	%RSD*
Serum	3.00	3.20	0.20	0.21 ± 0.17	5.00	0.17	0.21 ± 0.18	5.00	0. 26
	6.00	6.28	0.28	0.29 ± 0.15	3.50	0.16	0.29 ± 0.16	3.50	0. 17
	9.00	9.2	0.20	0.19 ± 0.19	5.00	0.12	0.21 ± 0.20	5.00	0. 13
Urine	3.00	3.21	0.21	0.21 ± 0.27	2.38	0.17	0.22 ± 0.28	4.70	0. 26
	6.00	6.21	0.21	0.22 ± 0.15	4.70	0.16	0.20 ± 0.17	4.70	0. 17
	9.00	9.19	0.19	0.20 ± 0.19	5.20	0.12	0.18 ± 0.19	5.20	0. 14
Hair	4.00	4.39	0.39	0.40 ± 0.10	2.50	0.21	0.41 ± 0.12	5.10	0. 22
	6.00	6.40	0.40	0.38 ± 0.25	5.00	0.15	0.39 ± 0.24	2.50	0. 16
	9.00	9.29	0.29	0.30 ± 0.12	3.40	0.12	0.31 ± 0.14	6.89	0. 14

*The values are multiplied by 10^{-7} mol L^{-1} for the proposed method.
%RE is the percent relative error, %RSD is the relative standard deviation and CL. Confidence limits were calculated from: CL = ± tS/√n. (The tabulated value of *t* is 4.303, at the 95% confidence level, S = standard deviation and n = number of measurements)

Table 4: Determination of tyrosine in serum, urine and hair samples by the proposed method using the nano optical sensor.

Sample	tyrosine added*	Total tyrosine found*	Actual tyrosine found*	Average*	(Average recovery ± %RSD)*
Serum	3.0	3.2	0.20	0.21	105.0 ± 0.37
	6.0	6.28	0.28	0.29	103.5 ± 0.16
	9.0	9.2	0.20	0.19	95.00 ±0.22
Urine	3.0	3.21	0.21	0.205	97.6 ± 0.27
	6.0	6.21	0.21	0.22	104.7 ± 0.26
	9.0	9.19	0.19	0.20	105.2 ±0.32
Hair	4.0	4.39	0.39	0.40	102.5 ±0.32
	6.0	6.4	0.40	0.38	95.00 ± 0.31
	9.0	9.29	0.29	0.30	103.4 ±0.41

*The values are multiplied by 10^{-7} mol L^{-1} for the proposed method.
The average of nine measurements.

A low cost and very sensitive method for the assessment of the tyrosine in blood serum, urine and hair samples was developed. The method was based upon measuring the luminescence intensity of the nano optical sensor Eu-Ciprofloxacin complex doped in sol-gel matrix at 617 nm and in presence of different concentrations of tyrosine in acetonitrile at (pH 9.1, λ_{ex}=380 nm). The significant quenching of the luminescence intensity of Eu-Ciprofloxacin complex by different concentrations of tyrosine was successfully used as an optical sensor in acetonitrile at 617 nm. The effect of different analytical parameters, e.g. pH, solvent, tyrosine concentration and foreign ions concentrations were fully accomplished.