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Research Article

NOVEL CHEMILUMINOMETRIC-FLUORESCENCE ENERGY TRANSFER (FRET) METHOD FOR THE DETERMINATION OF IRON (II) IN ORE ALLOYS USING GENERATED PRE-ABSORBED LUMINOL (DONOR MOLECULE) FROM POLY ACRYLIC ACID GEL BEADS BY CONTINUOUS FLOW INJECTION ANALYSIS

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ABSTRACT
Using a continuous flow injection analysis (CFIA), this work investigates a newly analytical method characterized by its speed and sensitivity for the determination of Fe(II) ion in ore alloys samples via the measurement of total luminescence which include the chemiluminescence and the created fluorescence by the in situ radiation of the released chemiluminescence emission using fluorescein sodium salt as a fluorophore molecule which excited by the generated chemiluminescence light (blue-violet λ=425 nm) to give an emission spectrum at λ=530 nm. Themethod is based on the oxidation of chemiluminescence donor molecule that loaded on poly acrylic acid gel beads (i.e. luminol solution 1×10^{-3}mol.L^{-1}) by hydrogen peroxide solution (5×10^{-5}mol.L^{-1}). Ferrous ion catalysts the CL-reaction and the energy of total emitted photons is measured and related to the concentration in a single well defined profile. The linearity for the range 0.005-20.0 µg.mL^{-1} has correlation coefficient 0.9909, and a coefficient of determination is 98.18% with a limit of detection (S/N =3) 16 pg/sample using 32µl of Fe(II) ion as a sample segment, while using quadratic regression gave a correlation coefficient for 0.005-20.0 µg.mL^{-1} of 0.9955, and a coefficient of determination 99.10%. Repeatability of 0.5 and 3.0 µg.mL^{-1}of Fe(II) ion solution (five successive injections) was < 1.0%. The method was applied satisfactorily for the determination of ferrous ion in two types of iron ore alloys using standard addition method.

Keywords: Chemiluminescence, in situ fluorescence, ferrous ion, iron ore alloy, PAA gel bead, CFIA.

1- INTRODUCTION

Iron is the most common element on earth, forming much of its outer and inner core[1]. Its normally does not occur as a free element in the earth, since most of iron in the crust is found combined with oxygen as iron oxide minerals such as hematite (Fe_2O_3) and magnetite (Fe_3O_4) and several grades of iron ores, such as laminate are found, also large deposits of iron are found in banded iron formations [2,3]. Iron is an absolute requirement for life including humans, plant and animals. An iron complex with the protein hemoglobin is necessary for oxygen transport in the blood[4]. All use iron which can be found in a wide variety of sources, it's also the major constituent in steel making and several of its oxides find use as paint pigments, polishing compounds, magnetic inks and coatings for magnetic tapes[5]. As iron is one of the most frequently determined analyte in environmental samples many methods have been developed for its determination[6,7] such as spectrophotometric[8,9], atomic absorption spectrophotometry[10], inductive coupled plasma[11], electroanalysis[12], fluorometry[13], and chemiluminescence methods proposed for determination of iron species in samples[14-17].

The present study describes a simple chemilumino-fluorometric FIA method that developed for the determination of Fe (II) ion in two types of ore alloys, the method based on the measurement of total luminescence light that released from Lu $_{GB}$-H_2O_2-Fe(II) ion system where the donor molecule (Luminol solution) is loaded on Poly acrylic acid gel beads.

2- Experimental

2-1- Chemicals and Reagents

All chemicals were used of analytical-reagent grade while distilled water was used to prepare the solutions. A standard solution of 1000 µg.mL^{-1} Iron (II) ion as ferrous ammonium sulphate $(NH_4)_2Fe(SO_4)_2.6H_2O$. (392.16 g.mol^{-1}, Showa Chemicals Co., Ltd. Tokyo, Japan) was prepared by dissolving 3.5112 g. in 500 mL distilled water, a few drops of concentrated sulfuric acid was added to the weighted amount in order to keep iron in the lowest oxidation state (i.e. Fe(II)).A stock solution (1×10^{-3} mol.L^{-1}) of Luminol solution (5-amino phthalylhydrazide) $C_8H_7N_3O_2$ (177.16 g.mol^{-1},BDH) was prepared by dissolving 0.0885g in 500 mL of 0.05mol.L^{-1} solution of sodium carbonate Na_2CO_3(105.97 g.mol^{-1}, BDH), prepared by dissolving 2.6493g in 500 mL distilled water. Stock solutions of hydrogen peroxide H2O2 (1×10-2mol.L-1) was prepared by pipetting 3.7 mL of hydrogen peroxide (20% vol., 34.01 g.mol-1, Romil LTD.) and complete the volume with distilled water to 500 mL volumetric flask. Hydrogen peroxide molarity was fixed in sulfuric acid medium (1:1) with potassium permanganate solution $KMnO_4$ (0.1mol.L^{-1}) (158.03g.mol^{-1}, Hopkin&William) was prepared by dissolving 7.9015g in 500 mL of distilled water. This solution was standardized previously against Sodium oxalate solution $Na_2C_2O_4$ 0.1 mol.L^{-1} (134.0g.mol^{-1}, BDH) prepared by dissolving 3.35g in 250 ml distilled water. A stock solution (2×10^{-3} mol.L^{-1}) of fluorescein free acid $C_{20}H_{12}O_5$ (332.31 g.mol^{-1}, BDH) prepared by dissolving 0.3323 g in 0.05 %NaOH then complete the volume with distilled water to 250 mL volumetric flask.

2-2- Preparation of Poly Acrylic Acid Gel Beads

Commercial poly acrylic acid gel beads (water crystal) were sorted according to weight and diameter. PAA gel beads that have weights ranging from 35.0-39.0 mg. were washed and swelled in distilled water, this process is a type of cleaning the interior as well as the whole gel bead in order to prepare PAA gel beads for work. Swelled gel beads were dried using a homemade drying cabinet. Dehydration process needs about 132 hours at 45°C and 8% relative humidity. Sorted gel beads were kept in clean and dry containers according to their weight, which corresponds with their diameter due to regular spherical shape. All these treatments were done in order to obtain and in turn use regular unbiased sample of poly acrylic acid gel beads.

2-3-Preparation of Samples Solutions (Iron Ore Alloys)

Two types of iron ore alloys were used as real samples (iron ore Northamptos-shire 302, 35.5% Fe and iron ore sinter 303, 35.9% Fe content, British chemical standards). A weight amount equivalent to 40 µg.mL^{-1} Fe(II) ion from each alloy was dissolved in 50 mL beaker using 5.0 mL of concentrated hydrochloric acid (38%, 1.1 g.mL^{-1}, BDH) then slow heating was done by warming up the solutions on an IR heater in order to complete dissolution until just about dryness then a pellet of zinc was added within no extra acid to each alloy solution followed by filtration through a filter paper to 250 mL volumetric flask and zinc pellets were kept inside the solution for maintaining iron in the lowest valency state (i.e. ferrous (II)). 7.5 mL from each alloy solution (dissolving product) was transferred to each of a series of 100 mL volumetric flask which were prepared for standard addition curve.

2-4- Apparatus

The flow system consist of variable speeds peristaltic pump- 4 channels (Switzerland) an Ismatic type ISM796. A rotary 6-port injection valve (Teflon) (Rheodyne, U.S.A.) with sample loop of 1mm i.d. Teflon, variable length. Electronic measuring system consist of photomultiplier tube PMT (Hama Matsu R372, Japan) enclosed with the chemiluminescence cell by a black leather in order to reduce the background interferences. DC voltage power supply (0-1.6 KV) type (JOBIN YVON- France). Dual detector (United Detector Technology, U.S.A.) capable of measuring pA-nA level. The read out of the system composed of x-t potentiometric recorder (1-500 mV) (KOMPENSO GRAPH C-1032) SIEMENS (Germany).

A special designed homemade gel bead cell unit was used in this work. This cell will be the measuring cell instantly with the released luminol solution from the gel beads. Figure (1) shows the three dimensional arrangement design of the gel bead cell unit (GBCU).

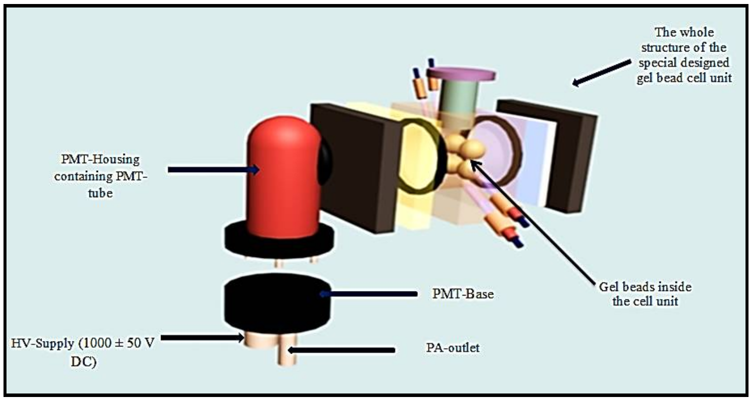

Fig. 1: Gel bead cell unit (GBCU) coupled with the photomultiplier (PMT)
****Random number of gel bead is shown (i.e. even lesser or more than four gel beads can be manipulated in the gel bead cell unit)**

2-5-Methodology

The whole manifold system for ferrous ion determination via Lu_{GB}-H_2O_2-Fe(II)-Fluorescein system is shown in Figure (2).

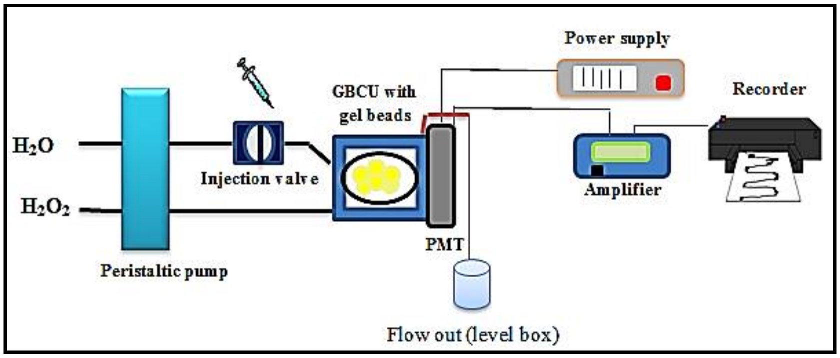

Fig. 2: Schematic diagram of FIA-CL system with the GBCU that used for Fe (II) determination

The manifold system is composed of two lines: first line supplies the carrier stream (distilled water) leading to the injection valve, which allows the use of 32µL and a flow rate of 2.0 mL.min^{-1}. The second line supplies hydrogen peroxide (5×10^{-5}mol.L^{-1}) at 2.65 mL.min^{-1}. Both out coming lines meet at the CL-reaction cell (i.e. GBCU) which retained 14 gel beads that were swelled previously (24 hours at least) in luminol solution (1×10^{-3}mol.l^{-1}) which diffused from within the gel beads surfaces to the surrounding environment where donor molecule (Luminol) is oxidized by hydrogen peroxide in alkaline medium in presence of Fe(II) ion and the emitted CL-light used to irradiate the fluorophore acceptor molecule (i.e. fluorescein sodium salt). The duration of injection period was 20 seconds for complete sample discharge from the injection valve; this was established while the CL-method. Two minutes as a time lag period was found to be necessary for releasing enough amount of luminol to conduct the CL-reaction inside the GBCU which is well protected with blacknon transparent leather, keeping both the PMT and the CL-cell in a close attachment. The obtained luminescence responses were assayed via the PMT, while the converted potential difference was recorded on x-t potentiometric recorder. Luminol CL-emission gives a maximum absorbance at 425 nm which absorbed by fluorescein molecule (fluorophore) to give a green band spectrum at 530-532 nm.

3- RESULTS AND DISCUSSION

3-1- Optimization of Fluorophore Molecule Concentration (Fluorescein Sodium Salt)

Series solutions of fluorescein salt were prepared for the range (1×10^{-5}-3×10^{-4} mol.L^{-1}) and mixed with constant concentration of ferrous ion. 32µL of Fe(II) ion was used as a sample volume on the carrier stream of distilled water. Table no.1 summarizes the obtained results, while Figure (3) shows peak profile for different concentrations of fluorescein sodium salt.

Table 1: Effect of fluorescein salt concentration on the in situ fluorescence emission expressed as average peak height (mV)

Conc. of Fluorescein salt (M)	CL. responses expressed as peak height (mV).	Average ($\bar{y}_i$) (mV)	Standard deviation(SD) σ_{n-1}	Repeatability %RSD=(SD/$\bar{y}_i$)* 100%	Confidence interval of the mean at 95% limit $\bar{y}_i \pm t_{(0.05/2),n-1} *(\sigma_{n-1}/\sqrt{n})$
1×10^{-5}	800, 805, 795	800	5.00	0.625	800 ± 12.423
3×10^{-5}	1003, 1000, 997	1000	3.00	0.300	1000 ± 7.453
5×10^{-5}	824, 825, 823	824	1.00	0.121	824 ± 2.483
1×10^{-4}	480, 476, 484	480	4.00	0.833	480 ± 9.936
3×10^{-4}	446, 455, 449	450	4.58	1.018	450 ± 11.377

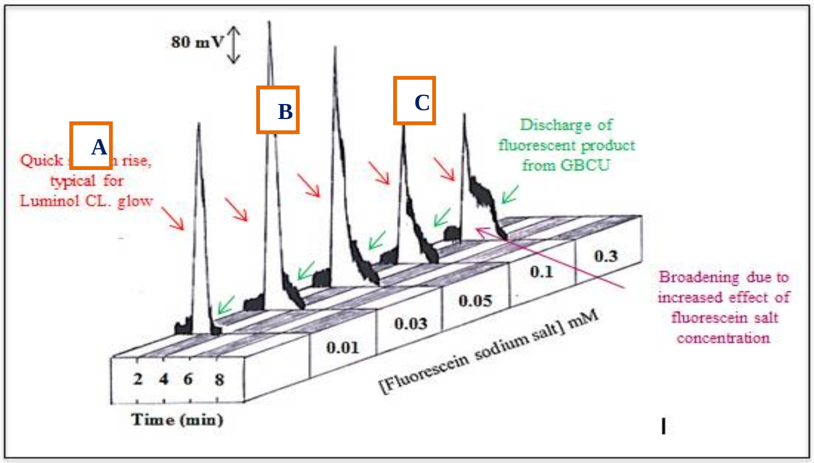

Fig. 3: Total Luminescence response-time peak profile for different concentrations of Fluorescein sodium salt

A: Single clear peak profile takes place in short time with no broadening.

B: The extended measured emission at the tail of the peak profile. The first sharp peak (red arrow) till near the end represents typical luminal CL-glow while the second part (green arrow) is characteristic of the excessive amount of fluorophore molecules at the GBCU.

C: The profile shows effect beginning of extended fluorescence at early stages compared to A&B this profile also takes longer time than A&B.

In Figure (4) which shows the variation of total Luminescence versus fluorescein salt concentration a sharp decrease in total luminescence response can be recognized at high concentrations of fluorescein salt, this might be attributed to the self-quenching inner filter effect of extended concentration of fluorophore molecules, coming up to a conclusion that: high concentration of fluorescein is not necessary to increase total light observed by the detector (PMT). Therefore; a compromisehas to be done in order to have a sharp response and without losing any emitted light whatever the emission is coming from.

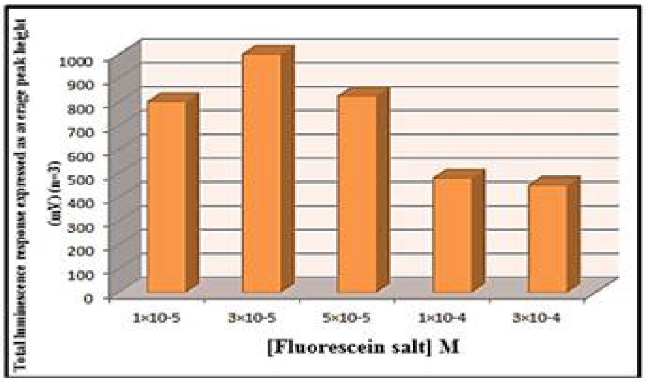

Fig. 4: Variation of total Luminescence response versus Fluorescein sodium salt concentration (expressed as average peak height mV)

In Figure (5) the dark shaded area represents the unnecessary and the effect of high concentrations of fluorophore molecule which leads to clear decrease in the obtained response.

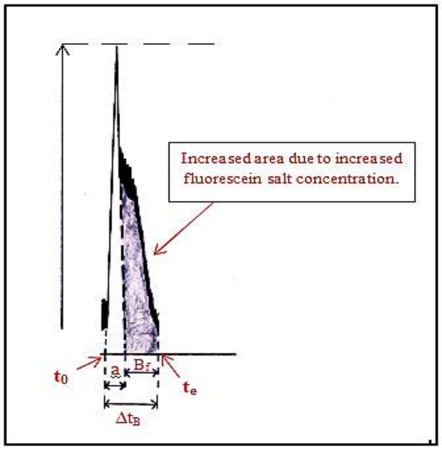

Fig. 5: Effect of high concentration of fluorescein sodium salt

*** $t_e - t_0 = \Delta t_B$**

$\Delta t_B - a = B_F$ (B_F quenching effect results from increment in fluorescein sodium salt)

In Figure (6) a plot of peak height/Δt_Bversus fluorescein salt concentration shows a sudden drop in the obtained response (i.e. peak height)with increasing in fluorophore concentration which represents self-quenching effect.

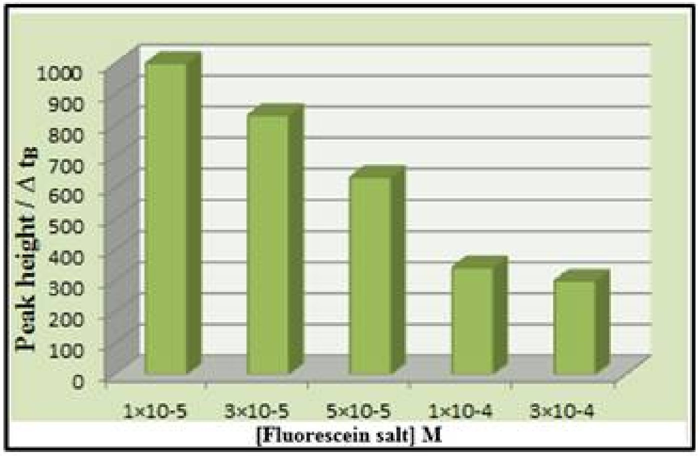

Fig. 6: Variation of total Luminescence response versus Fluorescein sodium salt concentration(expressed as Peak height/Δt_Bratio)

In Figure (7) a plot of B_f value versus Fluorescein sodium salt concentration shows the extended emission which results from unnecessary high concentration of the fluorophore molecules.

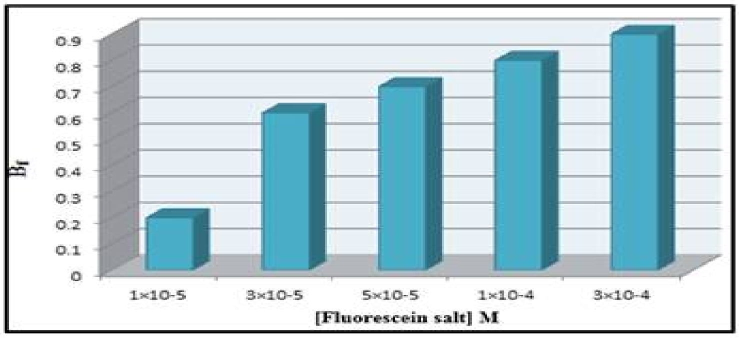

Fig. 7: Variation of total Luminescence response versus Fluorescein sodium salt concentration (expressed asB_f self-quenching effect)

3-2- Variation of Total Luminescence Response VersusIron (II) IonConcentration

A series of Fe(II) ion solutions (0.005-25 µg.mL^{-1}) were prepared in 3×10^{-5} mol.L^{-1}of fluorescein sodium salt solution. Acalibration graph was constructed for the variation of CL-response with Fe(II) ion concentration at the range of 0.005-20 µg.mL^{-1}.Table (2) tabulates all the obtained values including the average of three successive measurements, standard deviation, percentage relative standard deviation and confidence interval of the average obtained from linear regression analysis carried out for the data obtained using equation of the form: Response (mV) =intercept + slope [Fe(II) ion] µg.mL^{-1}.

Table 2: variation of total luminescence response versus the concentration of Fe(II) ion

[Fe (II) ion] µg.ml^{-1}	CL- responses expressed as Average peak height (mV). (n=3)	Standard deviation(SD) σ_{n-1}	Repeatability RSD%	Confidence interval of the mean at 95% confidence limit $\bar{y}_i \pm t_{(0.05/2),n-1} * (\sigma_{n-1}/\sqrt{n})$
0.005	201	1.732	0.862	201 ± 4.303
0.01	292	4.000	1.370	292 ± 9.936
0.1	445	4.583	1.030	445 ± 11.386
0.5	484	4.041	0.835	484 ± 10.039
1	751	2.646	0.352	751 ± 6.575
2	852	4.000	0.469	852 ± 9.936
3	1016	8.000	0.787	1016 ± 19.876
5	1509	4.163	0.276	1509 ± 0.684
7	1900	6.245	0.329	1900 ± 15.512
9	2094	2.000	0.096	2094 ± 4.970
11	2329	8.185	0.351	2329 ± 20.336
13	2563	5.196	0.203	2563 ± 12.909
15	2914	5.292	0.182	2914 ± 13.146
17	3085	4.359	0.141	3085 ± 10.831
20	3649	7.550	0.207	3649 ± 18.756
25	3600	17.436	0.484	3600 ± 43.318

Figure (8) shows the relationship between the total luminescence response expressed as average peak height and Fe(II) ion concentration.

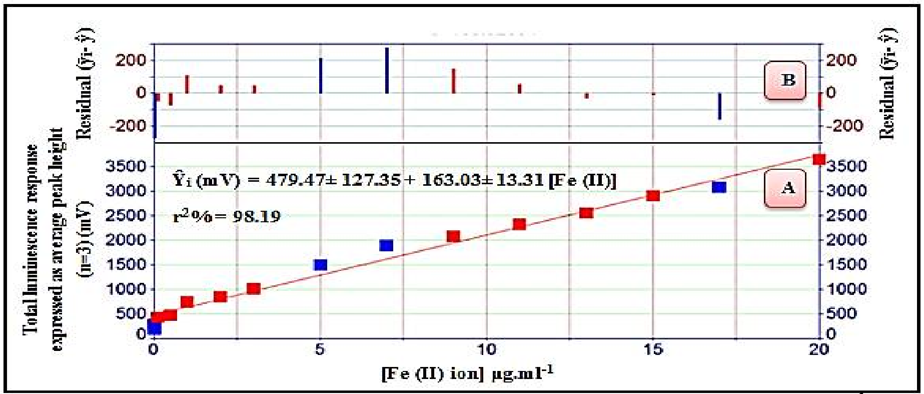

Fig. 8: Calibration graph for the variation of Fe(II) concentration (µg.mL^{-1}) on:
A: Total luminescence response expressed by linear equation,
B: Residual ($\bar{y}_i$– $\hat{Y}_i$) , $\bar{y}_i$: practical value ,$\hat{Y}_i$: estimated value

Data were also subjected to second degree equation of the form y= a+b[x] +c [x^2] as shown in Figure (9). The final output of the two equations forms, i.e. correlation coefficient,linearity percentage, and the calculated t-values at confidence interval of 95% are summarized in Table (3).

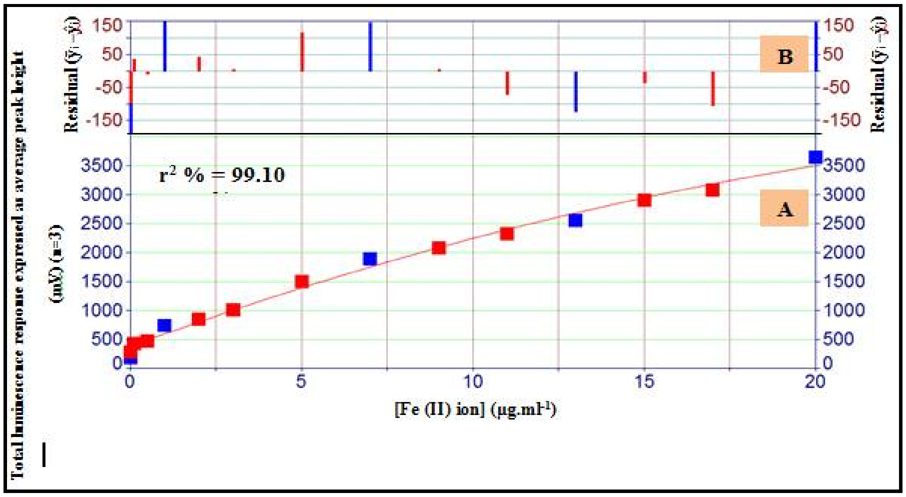

Fig. 9: A calibration graph for the relationship between Fe(II) ion concentration (µg.mL^{-1}) and CL- response using quadratic equation of the form y= a+b[x]+c [x]^2

Table 3: Summary of different equations forms for the variation of total luminescence response withFe(II) ion concentration (µg.ml^{-1})

Measured Conc. Of Fe(II)ion (µg.ml^{-1})	**Linear dynamic range (µg.ml^{-1}) (n=17)**	**Linear equation $\hat{Y}$i (mV) = a ± ts$_a$ +b ± ts$_b$ [Fe(II)] µg.ml^{-1} at confidence level 95% , n – 2**	**r r^2 r^2%**	**$t_{(0.05/2),15}$ at 95% confidence limit**	**t_{cal} $t = \frac{\lvert r \rvert \sqrt{n -}}{\sqrt{1 - r}}$**
0.005–25.0	0.005-20.0	479.47 ± 127.35 + 163.03 ± 13.31 [x]	0.9909 0.9818 98.18 %	2.160 << 26.468	
		Quadratic equation $\hat{Y}_i$(mV) = a ± ts$_a$ +b ± ts$_b$ [Fe(II)] + C ± ts$_c$[Fe(II)]^2 at confidence level 95% , n – 2			
		388.92± 108.48 + 216.24 ± 34.15 [x] - 3.03 ± (-6.54) [x]^2	0.9955 0.9910 99.10 %	2.16 << 37.787	

$\hat{Y}$i (mV) =Estimated CL-response for (n=3), [x] = [Fe (II)] µg.ml^{-1}, r = correlation coefficient, r^2% = linearity percentage.
$t_{tab=}$ $t_{0.05/2, n-2}$ at 95% confidence level.

Three different approaches for the study of detection limit ofFe(II) ion were used. Obtained results are tabulated in table no. (4) Using sample volume of 32.0 µL. Figure (10) shows total luminescence reponse time profile of the lowest detectable concentration of ferrous ion.

Table 4: Summery of limit of detection based on different approaches at 32 μL sample volume

Practically based on gradual dilution for minimum Concentration	Based on the value of slope theoretical based on slop value $X = 3S_B$ / slope	Linear equation $\hat{Y}i$ (mV) = Y_B+3S_B
16.0 pg./sample	1.632 ng./sample	2.891 ng./sample

S_B: standard deviation of blank solution repeated for 13 times. , X= value of L.O.D based on slope.
Y_B: average response for the blank solution (equivalent to intercept in straight line equation).

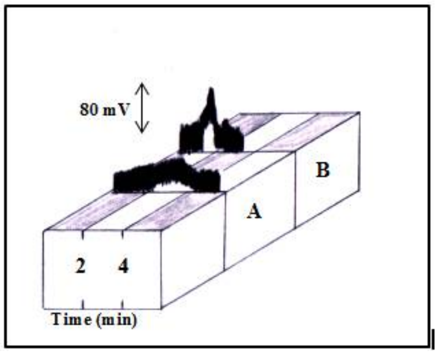

Fig. 10: peak time profile of A: distilled water, B: 0.0005 μg.ml^{-1} of Fe (II) ion

The values of RSD% for two selected concentrations of ferrous ion of five successive measurements as shown in figure (11 A, B) are tabulated in table no. (5).

Table 5: Repeatability results of Fe(II) ion

[Fe(II)] μg.ml^{-1}	no. of injection	CL- response expressed as average peak height (mV)	Standard deviation σ_{n-1}	%RSD	Confidence interval of the mean at 95% $\bar{y}_i \pm t_{0.05/2,\, n-1}\, \sigma_{n-1} / \sqrt{n}$
0.5	5	481.4	4.278	0.889	481.4 ± 5.310
3.0	5	1017	5.916	0.582	1017 ± 7.345

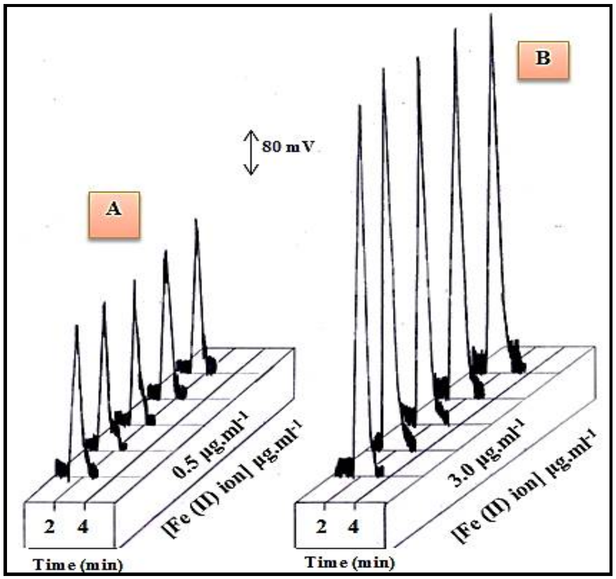

Fig. 11: The total luminescence response-time Profile for five successive repeatable measurements of Fe (II) ion; A: 0.5 μg.ml^{-1}, B: 3.0 μg.ml^{-1}

Application

The chemilumino-fluorometric FIA method achieved in this work was used for the determination of iron in two different iron ore alloys. The standard addition method was applied by preparing a series of solutions via transferring 7.5 mL of each alloy dissolution product to each of 8 volumetric flasks(100 mL), followed by addition of gradual volumes of standard Fe(II) ion solution (100 µg.mL^{-1}) ranging from 0.0-10.0µg.mL^{-1} in order to have the concentration range from 0.0 to 10.0 µg.mL^{-1} for the preparation of standard addition curve. Results were mathematically treated for standard addition method and they are tabulated in Table 6 and 7.

Table 6: Summary of linear regression equation of estimating Fe(II) ion by Luminol$_{G.B.}$-H_2O_2-Fe(II)ion (standard addition method)

Type of alloy sample	Range of Fe (II)ion conc. (µg.ml^{-1})	No.of measure-ements (n)	Linear equation $\hat{y}i$ (mV) = a ± ts$_a$ +b ± ts$_b$ [Fe(II)] µg.ml^{-1} at confidence level 95% , n – 2	r r^2 r^2%	$t_{(0.05/2),6}$ at 95% confidence limit	$t_{cal} = \frac{\lvert r\rvert\sqrt{n-2}}{\sqrt{1-r^2}}$
Iron ore 302	0-10	8	691.77± 369.15 +231.71± 64.11 [x]	0.9637 0.9287 92.87	2.447 < 8.839	
Iron ore 303			840.58 ± 237.01 +277.73 ± 41.16 [x]	0.9892 0.9785 97.85	2.447 < 16.525	

$\hat{Y}i$ (mV) =Estimated CL-response for (n=3), [x] = [Fe (II)] µg.ml^{-1}, r = correlation coefficient, r^2% = linearity percentage. $t_{tab}= t_{0.05/2, n-2}$ at 95% confidence level.

Table 7: Iron (II) ion determination in two types of iron ore alloys (302,303) by total luminescence of Luminol$_{G.B.}$- H_2O_2- Fe (II) ion system by standard addition method

Type of sample alloy	Wt. of sample (g.)	Wt. of Fe in 100 g. sample (theoretically)	Wt. of Fe (II) ion in sample alloy (g.)	[Fe(II)] ion µg.ml^{-1}	[Fe(II)]µg.mL^{-1} after dilution 7.5mL/100 mL (Theoretically)	[Fe(II)] µg.mL^{-1} (practically)	Wt. of Fe in 100 g. sample (practically)	Recovery %
302	0.0282	35.5	0.0100 theoretically 9.952×10^{-3} practically	40.0	3.000	2.9855	35.329	99.52 %
303	0.0279	35.9	0.0100 theoretically 10.089×10^{-3} practically			3.0266	36.2186	100.89

CONCLUSION

The proposed FI-Chemilumino-fluorometric method has a simple, rapid, inexpensive and high sensitivity for the determination of Fe(II) ion in two types of iron ore alloy samples based on the Luminol$_{G.B.}$- H_2O_2-Fe(II) ion system. the detection limit of the proposed method is 16 pg/sample and the RSD% less than 1.0%, also good agreement were observed for all samples, which is an indication of satisfactory accuracy of the proposed method, and can be used as an alternative method for determination of iron in iron ore alloys at this extreme level of sensitivity and high accepted trust ability.

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