

NEW CHEMILUMINOMETRIC CONTINUOUS FLOW INJECTION ANALYSIS FOR PRE-ABSORBED LUMINOL IN POLY ACRYLIC ACID GEL BEADS AND IT'S USE IN THE DETERMINATION OF IRON IN AN IRON ORE ALLOYS

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ABSTRACT

A flow injection system was combined with chemiluminescence detection for the on-line ultra-trace determination of iron (II) ion in its ore alloys in a sensitive, fast, and accurate method. The method was based on the oxidation of donor molecule (Luminol) which is loaded on poly acrylic acid gel beads by hydrogen peroxide in presence of Fe(II) ion as a chemiluminescence catalyst. Linear dynamic range for the chemiluminescence-emission versus Fe(II) ion concentration was (0.01-25.0 $\mu\text{g}.\text{ml}^{-1}$) while L.O.D. (S/N =3) was 32.0 pg/sample for the step wise dilution of the minimum concentration in the linear dynamic range of the calibration graph. The correlation coefficient r was 0.9736 while the percentage linearity $r^2\%$ was 94.79% and the relative standard deviation RSD% for 9 $\mu\text{g}.\text{ml}^{-1}$ of Fe(II) ion solution is 0.373% ($n=5$). The method was applied successfully for determination of iron (II) ion in two types of ore alloys (iron ore Northampton-shire 35.5% Fe and iron ore sinter 35.9% Fe) according to British chemical standards.

Keywords: chemiluminescence, flow injection analysis (FIA), Fe(II) ion, Iron ore alloys, gel beads.

1. INTRODUCTION

Iron is a silvery-white or grayish hard, brittle, fairly fusible metal, it is considered the fourth most abundant element in earth's crust and its abundance is estimated to be about 5%. Iron is found in two oxidation states ferrous (II) and ferric (III)¹. The most common ore of iron is hematite that appears as black sand on beaches or black seams when exposed in the ground. Iron ores (ferric oxides) also vary in color from brownish red to brick red to cherry red with a metallic shine. The pure metal is very reactive chemically and rapidly corrodes, especially in moist air or at elevated temperature to form rust; also it's highly reactive with most acids, releasing hydrogen from these acids². Iron is a vital constituent of plant and animal life, Since an adult human body has a typical iron content of approximately 4.0 g. and about 50% at which is found in hemoglobin so it's one of the most important essential elements and its deficiency or overload may cause health problems^{3,4}. The determination of iron in analytical chemistry has become a routine procedure because of its importance in normal living life⁵. There are many techniques reported for the determination of iron in literatures, some of the most commonly used methods include spectrophotometry⁶⁻⁹, atomic absorption spectrophotometry^{10,11}, inductive coupled plasma-mass spectrometry¹², and chemiluminescence methods¹³⁻¹⁹. This work describes a simple chemiluminometric-flow injection analysis method that has been developed for the determination of Fe (II) ion in alloys. The method based on the catalytic effect of the metal ion on the chemiluminescence reaction of $\text{Lu}_{\text{GB}}\text{-H}_2\text{O}_2\text{-Fe(II)}$ ion system where the donor molecule (Luminol) is loaded on poly acrylic acid gel beads (water crystals) which retained in homemade special designed gel bead cell unit (GBCU).

2. Experimental

Chemicals

All chemicals were used of analytical-reagent grade while distilled water was used to prepare the solutions. A standard solution of $1000 \mu\text{g}.\text{ml}^{-1}$ Iron (II) ion as ferrous ammonium sulphate $(\text{NH}_4)_2\text{Fe}(\text{SO}_4)_2 \cdot 6\text{H}_2\text{O}$ ($392.16 \text{ g}.\text{mol}^{-1}$, 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. $\text{Fe}(\text{II})$)²⁰. A stock solution ($1 \times 10^{-3} \text{ mol}.\text{L}^{-1}$) of Luminol solution (5-amino phthalylhydrazide) $\text{C}_8\text{H}_7\text{N}_3\text{O}_2$ ($177.16 \text{ g}.\text{mol}^{-1}$, BDH) was prepared by dissolving 0.0885g in 500 ml of $0.05 \text{ mol}.\text{L}^{-1}$ solution of sodium carbonate Na_2CO_3 ($105.97 \text{ g}.\text{mol}^{-1}$, BDH), prepared by dissolving 2.6493g in 500 ml distilled water. A stock solution of hydrogen peroxide H_2O_2 ($1 \times 10^{-2} \text{ mol}.\text{L}^{-1}$) was prepared by pipetting 3.7 ml of hydrogen peroxide (20% vol., $34.01 \text{ g}.\text{mol}^{-1}$, 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 ($0.1 \text{ mol}.\text{L}^{-1}$) ($158.03 \text{ g}.\text{mol}^{-1}$, Hopkin&William) was prepared by dissolving 7.9015g in 500 ml of distilled water. This solution was standardized previously against Sodium oxalate solution $\text{Na}_2\text{C}_2\text{O}_4$ $0.1 \text{ mol}.\text{L}^{-1}$ ($134.0 \text{ g}.\text{mol}^{-1}$, BDH) prepared by dissolving 3.35g in 250 ml distilled water.

Preparation of poly acrylic acid gel beads (water crystals)

Gel beads that are having weight range between 35.0-39.0mg, were washed and swelled in distilled water then dried using homemade drying cabinet Figure 1.

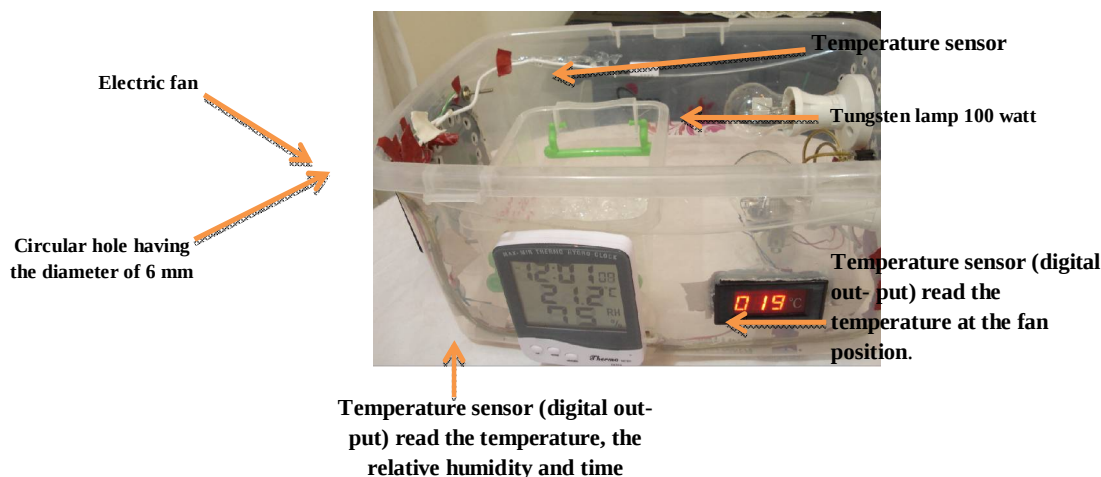


Fig. 1: homemade drying cabinet used for gel beads drying

The dehydration process need about 132 hours at 45°C and relative humidity 8%. The sorted gel beads kept in clean and dry containers based on their weight; which corresponds with diameter. All these treatments of gel beads were made in order to obtain and in turn use regular unbiased sample of poly acrylic beads.

Preparation of samples solutions (iron ore alloys)

Two types of iron ore alloys were used as real samples (iron ore Northamptonshire 302, 35.5% Fe and iron ore sinter 303, 35.9% Fe content, British chemical standards). A weight amount equivalent to $40 \mu\text{g}.\text{ml}^{-1}\text{Fe}(\text{II})$ ion from each alloy was dissolved in 50 ml beaker using 5.0 ml of concentrated hydrochloric acid (38%, $1.1 \text{ g}.\text{ml}^{-1}$, 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.

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). Figure 2 shows the homemade cell unit which was used for the reaction, mixing, and measuring purposes since its retained gel beads that were loaded with luminol solution which is the chemiluminescence donor reagent.

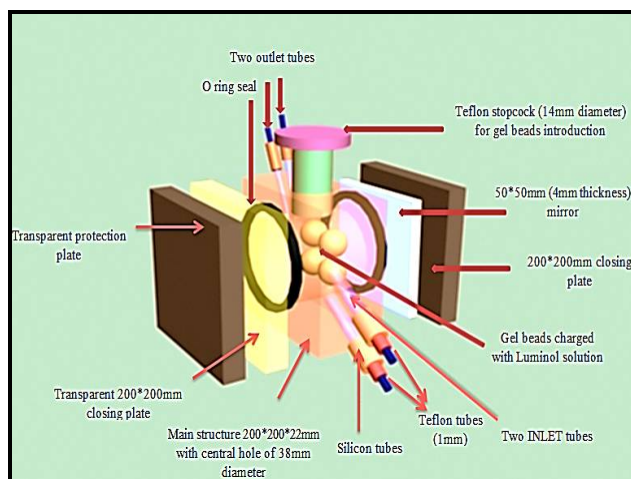


Fig. 2: 3D-structural design of the gel bead cell unit with two inlets and two outlets used in the determination of metal ion via flow through CL-reaction.Random number of gel bead is shown (i.e. even lesser or more than four gel beads can be manipulated in the gel bead cell unit.)**

3. Methodology

The flow system that used for the determination of ferrous ion by Luminol_{GB}-H₂O₂-Fe(II) ion system shown schematically in Figure 3.

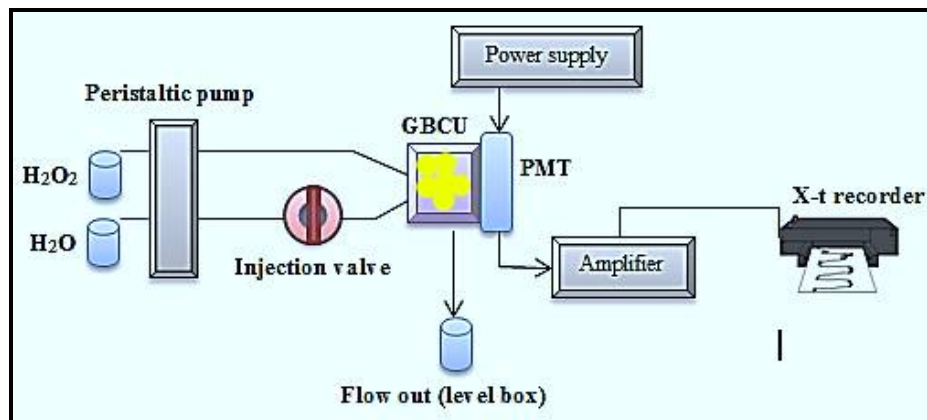


Fig. 3: Schematic diagram of the CFIA system with GBCU that used for determination of Fe(II) ion

The flow system composed from two lines: the first one supply hydrogen peroxide solution $5 \times 10^{-5} \text{ mol.L}^{-1}$ at 2.65 ml.min^{-1} flow rate; while the second line is the carrier stream (distilled water) which leads to the injection valve to carry Fe(II) ion sample segment of $32 \mu\text{L}$ at 2.0 ml.min^{-1} flow rate and using 20 seconds time to discharge this segment from the injection valve loop. Both out coming lines meet and mixed at the special designed cell unit with the donor reagent (Luminol) which is diffused from within the surfaces of fourteen poly acrylic acid gel beads to the surrounding environment, these gel beads were saturated previously with Luminol solution ($3 \times 10^{-3} \text{ mol.L}^{-1}$) by passing the

chemiluminescence donor solution in a close circular flow from the pump to the cell returning back the outlet to the main supplier beaker.

Two minutes as a recharging build up time was left between each two successive measurements in order to allow enough amount of luminol to diffuse to the GBCU environment where oxidized by hydrogen peroxide in presence of Fe(II) ion to produce a flash of light which is picked up by Uv-Vis-NIR PMT. The reaction has to occur in a completely dark chamber to prevent outside light from reaching the PMT, and the obtained signals are processed to read either digitally in pA, nA, μ A or responses are recorded on x-t potentiometric recorder at variable ranged based on the obtained signals. Figure 4 shows the preliminary CL-response-time profile for three different concentrations of Fe (II) ion.

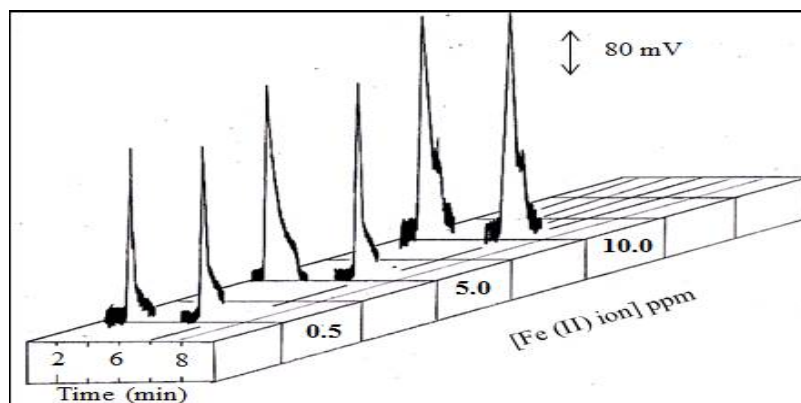
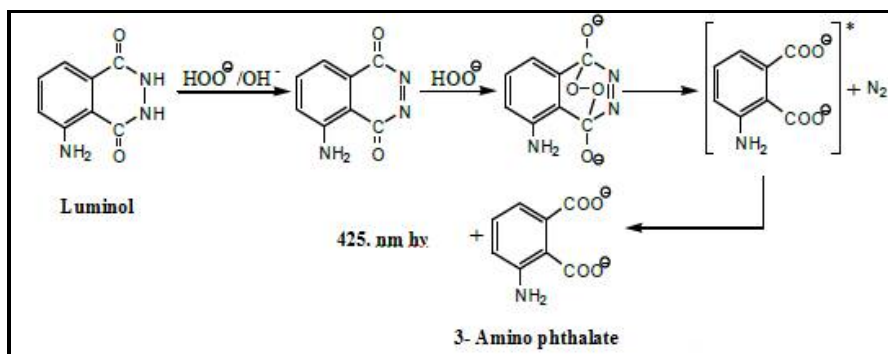


Fig. 4: Preliminary CL-responses-time profile for different concentrations of Fe(II) ion (ppm) at randomly selected reaction parameters*. ** 5×10^{-5} mol.L⁻¹ H₂O₂, 1×10^{-3} mol.L⁻¹ Luminol, 600 VDC applied photo cathode voltage, 2.0 min. recharging build up time, 2.0 ml.min⁻¹ flow rate and open valve mode (75 sec.) as a purge time

4. Mechanism of reaction

A generalized reaction mechanism found throughout the literature for Luminol shown in scheme no.1^{21, 22}.



Scheme. 1: The general reaction of Luminol to produce the chemiluminescence emission. Fe(II) catalyzes the second step in this reaction scheme. The light emitted after the third step is proportional to [Fe (II) ion] within a certain concentration range

5. Results and discussion

Optimization of experimental conditions

A series of experiments were conducted to establish the optimum parameters. Chemical variables such as reagents concentration, as well as physical variables including flow rate, purge time, recharging build up time and injected sample volume were investigated.

Chemical variables

Effect of hydrogen peroxide concentration

A series of hydrogen peroxide solutions (1×10^{-5} – 5×10^{-4} mol.L⁻¹) were prepared and by using the preliminary conditions, 3 μ g.ml⁻¹ of Fe(II) ion, sample volume of 40 μ L on the carrier stream (distilled

water) at a flow rate of 2.0, 2.65 ml.min⁻¹ for both carrier and hydrogen peroxide line respectively within using 2.0 min as a waiting time and open valve mode (i.e. 75 sec.) as a purge time with 600 VDC as applied photocathode voltage. Each measurement was repeated for three successive times. Table 1 tabulates all obtained results and Figure (5 A,B) shows that 5×10⁻⁵ mol.L⁻¹ was the optimum concentration.

Table 1: Effect of [H₂O₂] on the CL-response expressed as average peak height (mV) (n=3)

[H ₂ O ₂] Mol.L ⁻¹	CL-response expressed as peak height (n=3), (mV)	Average $\bar{y}_i$ (mV)	Standard deviation σ_{n-1}	RSD%	Confidence interval of the mean at 95% $\bar{y}_i \pm t_{0.05/2, n-1} \sigma_{n-1} / \sqrt{n}$
1 × 10 ⁻⁵	1088, 1086, 1090	1088.00	2.000	0.184	1088.00 ± 4.970
3 × 10 ⁻⁵	1232, 1240, 1232	1234.67	4.619	0.374	1234.67 ± 11.476
5 × 10 ⁻⁵	1720, 1718, 1725	1721.00	3.606	0.209	1721.00 ± 8.959
1 × 10 ⁻⁴	1060, 1052, 1060	1057.30	4.619	0.437	1057.30 ± 11.476
3 × 10 ⁻⁴	920, 920, 920	920.00	0.000	0.000	920.00 ± 0.000
5 × 10 ⁻⁴	808, 824, 800	810.67	12.220	1.507	810.67 ± 30.358

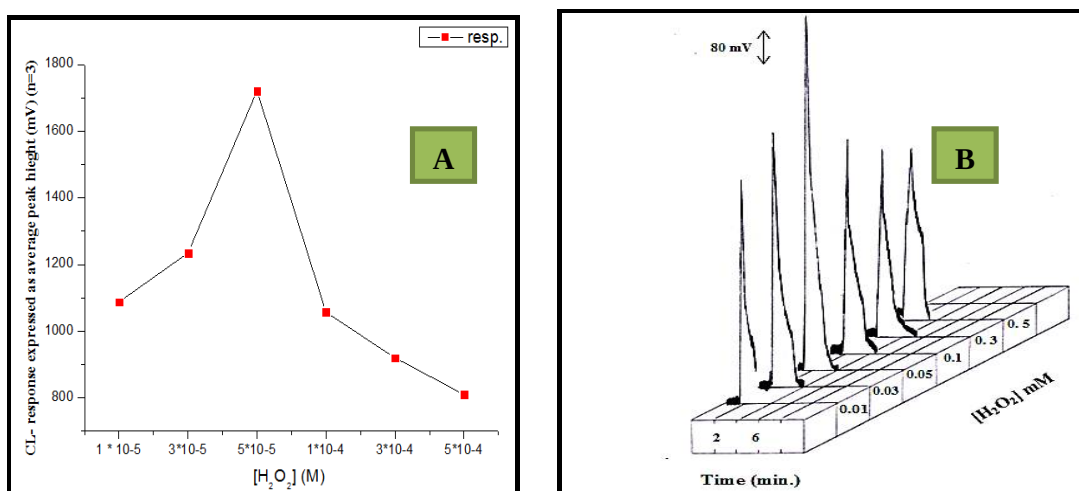


Fig. 5: Effect of [H₂O₂] on A- CL-response expressed as average peak height (mV) (n=3), B- CL- response- time profile

Physical variables

Effect of flow rate

Using fixed parameters which include 2.0 min. as a recharging build up time with an open valve mode (75 sec.) as a purge time for 40 μL of 3.0 μg.ml⁻¹ Fe(II) ion injected sample segment with 600 VDC applied photocathode voltage, and the optimum concentration of H₂O₂ 5×10⁻⁵ mol.L⁻¹. Optimum flow rate was conducted using variable flow rates ranged from 0.6-2.6 ml.min⁻¹ for carrier stream. Table 2 shows the obtained data while Figure (6 A,B) shows that an increase in CL-responses with increasing in flow rates in a linear increment without reaching a constant emission level, therefore; regarding the profile obtained and measureable signal in constant pattern 2.0 ml.min⁻¹ was chosen as an optimum flow rate.

Table 2: Effect of flow rate variation on CL-response (mV)

Pump speed indication approximate	Flow rate (ml.min ⁻¹)		Average CL-response expressed as peak height (n=3), $\bar{y}_i$ (mV)	RSD%	Confidence interval of the mean at 95% $\bar{y}_i \pm t_{0.05/2, n-1} \sigma_{n-1} / \sqrt{n}$
	Carrier Stream line	H ₂ O ₂ stream line			
10	0.6	0.9	324.0	1.235	324.0 ± 3.068
15	1.0	1.4	536.0	0.646	536.0 ± 8.608
20	1.2	1.8	848.0	0.312	848.0 ± 6.575
25	1.6	2.2	1000	0.436	1000 ± 10.831
30	2.0	2.65	1464	0.137	1464 ± 4.970
35	2.2	3.1	2595	0.168	2595 ± 10.831
40	2.6	3.5	2806.67	0.082	2806.67 ± 5.736

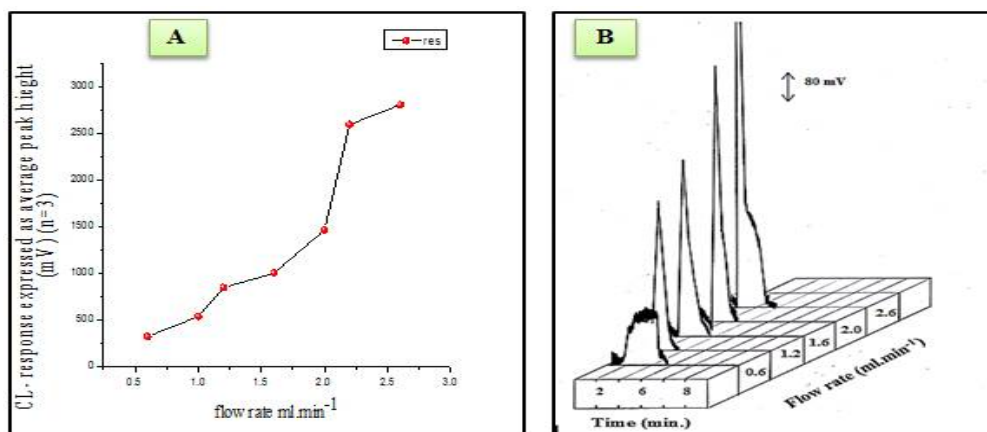


Fig. 6: Effect of variation of the flow rate on A- CL-response expressed as average peak height (n=3) (mV), B- CL-response-time profile

Effect of purge time

Using different purge times for sample segment using 5.0 sec.-75.0 sec.(open valve mode) allowed times for the carrier stream to pass through the injection valve, followed by turning the injection valve to the load mode. Using the optimum parameters, 2.0 ml.min⁻¹ flow rate, 5×10^{-5} mol.L⁻¹, 600 VDC applied voltage and 40 μ L of 3 μ g.ml⁻¹ Fe(II) ion with 2.0 min. as a recharging build up time. Table 3 sums up results. Unexpectedly between 20 sec. purge time up to open valve mode a decrease in CL-responses was occur at 30 and 40 sec. followed by clear increase, this could be attributed to the mode of valve operation (load/inject) mode which affect the peak maxima of the CL-response. Its expect that above open valve mode (75 sec.) a steady state could be achieved and this is shown in Figure (7 A, B).

Table 3: Effect of variation of purge time on CL-response (mV)

purge time (sec.)	CL-. response expressed as average peak height, $\bar{y}_i$, (n=3) (mV)	RSD%	Confidence interval of the mean at 95% $\bar{y}_i \pm t_{0.05/2, n-1} \sigma_{n-1} / \sqrt{n}$
5	570.67	0.405	570.67 $\pm$ 5.736
10	808.0	0.990	808.0 $\pm$ 19.876
15	1165	0.310	1165 $\pm$ 8.959
20	1602	0.125	1602 $\pm$ 4.970
30	1240	0.161	1240 $\pm$ 4.970
40	1213.33	0.381	1213.33 $\pm$ 11.467
50	1321	0.131	1321 $\pm$ 4.303
Open valve mode	1483	0.294	1483 $\pm$ 10.831

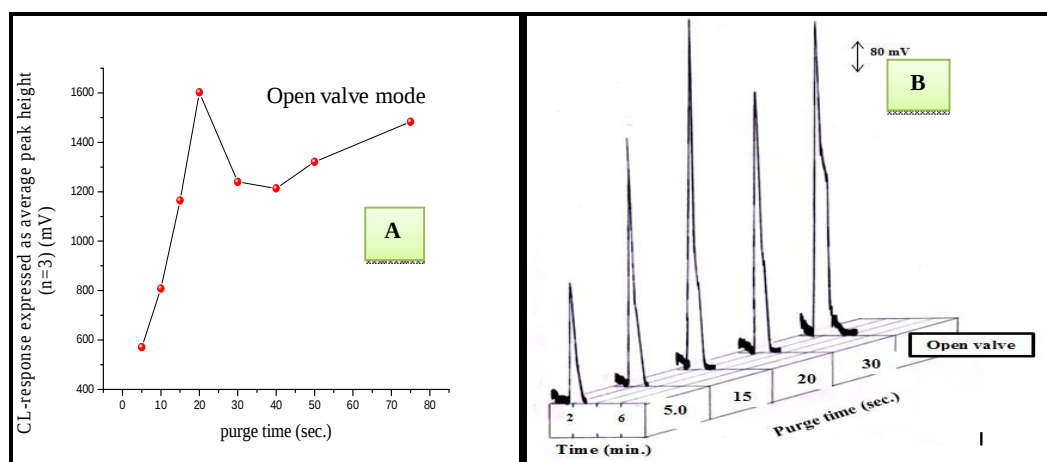


Fig. 7: Effect of purge time on the: A- CL-response expressed as average peak height (n=3), B- CL-response-time profile.

Effect of recharging build up time

A study was conducted to fix the optimum recharging build up time at stop-go mode of operation using the optimum parameters that were achieved in previous sections. Various waiting times ranging from 0.0 up to 5.0 minutes were used in which the pump in OFF position i.e. allowing enough time for Luminol solution to be diffused out through the 14 gel beads surfaces leaving the environment surrounding the gel beads with satisfactory concentration of luminol to conduct the CL- reaction. Table 4 list all the waiting times that were tried. Figure (8 A,B) shows a nearly linear increase in CL-responses which is expected, therefore; a compromise is made between number of injections with the stability of the obtained response so, 2.0 min. was found to be satisfied for use in this research work.

Table 4: Effect of recharging builds up time on CL-response (mV)

Off pumping time(min.)	Average of CL. responses expressed as average peak height, $\bar{y}_i$, (n=3) (mV)	RSD%	Confidence interval of the mean at 95% $\bar{y}_i \pm t_{0.05/2, n-1} \sigma_{n-1} / \sqrt{n}$
0	208.0	0.962	208.0 $\pm$ 4.970
0.5	448.0	0.773	448.0 $\pm$ 8.606
1	632.0	0.316	632.0 $\pm$ 4.970
2	1304	0.406	1304 $\pm$ 13.146
3	1551.33	0.521	1551.33 $\pm$ 20.082
4	1790.33	0.475	1790.33 $\pm$ 21.132
5	2544	0.068	2544 $\pm$ 4.303

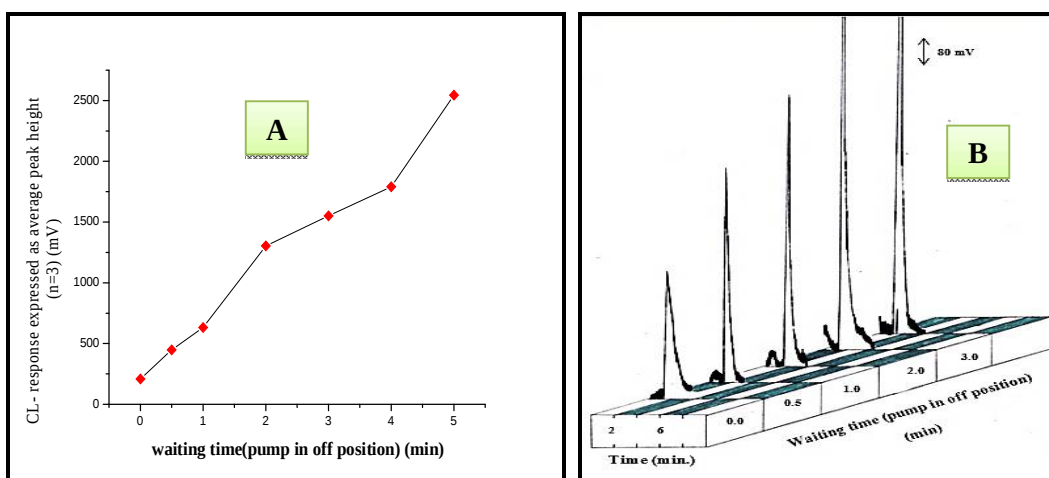


Fig. 8: Effect of recharging build up time on: -A- CL-response expressed as average peak height (mV) (n=3),-B- CL-response-time profile

Effect of sample volume

In order to establish the optimum sample volume, a study was conducted using variable sample volumes (8.0-55 μL). Table no. 5 sums up all volumes that were tried by using $5 \times 10^{-5} \text{ mol.L}^{-1} \text{H}_2\text{O}_2$, 2.0 ml.min^{-1} flow rate for carrier stream, 600 VDC applied voltage, 2.0 min. as a waiting time and 20 seconds as a purge time for $3.0 \mu\text{g.ml}^{-1} \text{Fe(II)}$ ion sample. Figure (8 A,B) shows that an increase in the injected sample volume leads to increase in CL-response height without affecting the profile in general since sample volume reflects the concentration of the complementary reactant that is necessary in CL- reaction (i.e. Fe(II) ion) therefore; $32.0 \mu\text{L}$ was used throughout this work.

Table 5: Effect of sample volume variation on CL-response

Loop length (cm)	Injected sample volume (μL)	CL-. response expressed as average peak height, $\bar{y}_i$, (n=3) (mV)	RSD%	Confidence interval of the mean at 95% $\bar{y}_i \pm t_{0.05/2, n-1} \sigma_{n-1} / \sqrt{n}$
1	8	660.0	0.401	660.0 $\pm$ 6.575
2	16	744.0	0.134	744.0 $\pm$ 2.483
3	25	1104	0.181	1104 $\pm$ 4.970
4	32	1176	0.00	1176 $\pm$ 0.00
5	40	1382	0.145	1382 $\pm$ 4.970
6	47	2178.67	0.106	2178.67 $\pm$ 5.736
7	55	2376.0	0.344	2376.0 $\pm$ 20.336

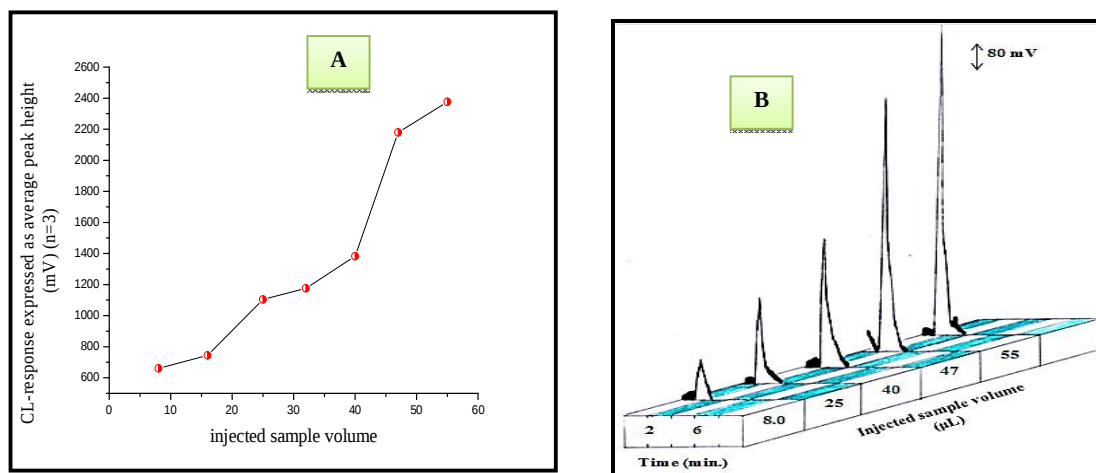


Fig. 8: Effect of variation of sample volume on: A- CL-response expressed as average peak height (mV) (n=3), B- CL-response -time profile

Calibration graph

A series of iron (II) solutions ($0.01\text{--}35.0 \mu\text{g.mL}^{-1}$) were prepared for the purpose of using them for a scatter plot diagram followed by the choice of calibration graph. CL-response of average peak height (mV) (n=3) was plotted against Fe(II) ion concentration, a straight graph from $0.01\text{--}25.0 \mu\text{g.mL}^{-1}$ Fe (II) ion was obtained. All the obtained results were tabulated in table 6, while their representation was shown in Figure (9 A, B).

Table 6: Summary of different equations forms for the variation of CL-response with Fe (II) ion concentration ($\mu\text{g.mL}^{-1}$)

Measured Conc. Of Fe (II) ion ($\mu\text{g.mL}^{-1}$)	Linear dynamic range ($\mu\text{g.mL}^{-1}$) (n=17)	Linear equation $\hat{Y}_i \text{ (mV)} = a \pm ts_a + b \pm ts_b [\text{Fe(II)}] \mu\text{g.mL}^{-1}$ at confidence level 95%, n - 2	r , r^2 $r^2\%$	$t_{(0.05/2), 15}$ at 95% confidence limit	$t_{\text{cal}} = \frac{ \bar{y}_i \sqrt{n-2}}{\sqrt{1-r^2}}$
0.01-35.0	0.01-25.0	$361.96 \pm 160.43 + 114.28 \pm 14.74 [\text{Fe(II)}]$	0.9736 0.9479 94.79%	2.131	<< 16.538

$\hat{Y}_i$ (mV) = Estimated CL-response for (n=3), r = correlation coefficient, $r^2\%$ = linearity percentage. $t_{\text{tab}} = t_{0.05/2, n-2}$ at 95% confidence level.

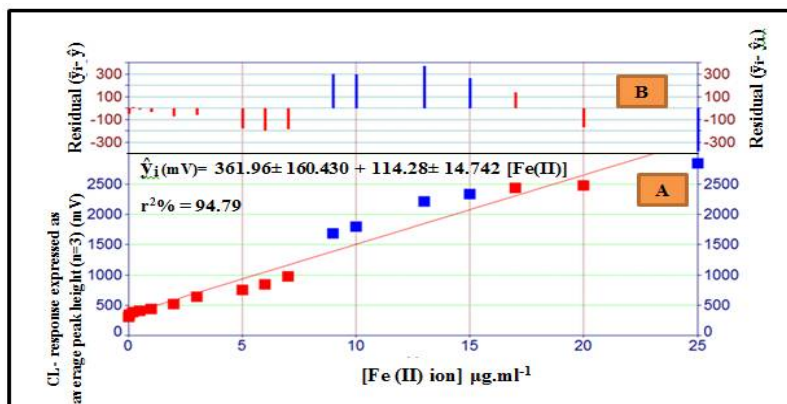


Fig. 9: Calibration graph for the variation of Fe (II) concentration ($\mu\text{g.ml}^{-1}$) on - A: CL- response expressed by linear equation, B-residual ($\hat{y}_i - \hat{y}_i$), $\hat{y}_i$: practical value, $\hat{y}_i$: estimated value

Using three different ways for the study of detection limit for Fe (II) ion. Obtained results are tabulated in table 7 using sample volume of 32.0 μL .

Table 7: Summary 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 $X = 3S_B / \text{slope}$	Linear equation $\hat{Y}_i (\text{mV}) = Y_B + 3S_B$
32.0 pg./sample	2.752 ng./sample	180 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).

Repeatability

This study was conducted for the determination of iron (II) ion via the measurements of the emitted light by $\text{Lu}_{\text{GB}}^- \text{H}_2\text{O}_2\text{-Fe(II)}$ ion CL-system at concentrations of 1.0 and 9.0 $\mu\text{g.ml}^{-1}$ of five successive measurements as shown in Figure (10 A,B). All the obtained results were sum up in Table 8.

Table 8: repeatability results of Fe (II) ion at optimum parameters by CFIA-CL method

[Fe(II)] $\mu\text{g.ml}^{-1}$	no. of injection	CL- response expressed as average peak height (mV)	Standard deviation σ_{n-1}	RSD %	Confidence interval of the mean at 95% $\bar{y}_i \pm t_{0.05/2, n-1} \sigma_{n-1} / \sqrt{n}$
1.0	5	456.80	5.933	1.299	456.80 ± 7.365
9.0	5	1426.40	5.367	0.373	1426.4 ± 6.662

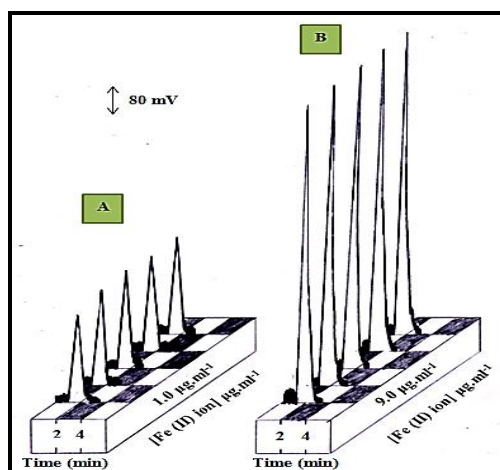


Fig. 10: The CL-response-time profile for five successive repeatable measurements of Fe(II) ion; A-1.0 $\mu\text{g.ml}^{-1}$, B- 9.0 $\mu\text{g.ml}^{-1}$

Applications

The chemiluminometric- 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 13 volumetric flasks(100 ml), followed by addition of gradual volumes of standard Fe(II) ion solution ($100 \mu\text{g}.\text{ml}^{-1}$) ranging from 0.0-20.0 $\mu\text{g}.\text{ml}^{-1}$ in order to have the concentration range from 0.0 to 20.0 $\mu\text{g}.\text{ml}^{-1}$ for the preparation of standard addition curve. Results were mathematically treated for standard addition method and they are tabulated in Table 9 and 10.

Table 9: Summary of linear regression equation of estimating Fe(II) ion by Luminol_{G.B.}-H₂O₂- Fe(II) ion (standard addition method)

Type of alloy sample	Range of Fe(II) ion conc. ($\mu\text{g}.\text{ml}^{-1}$)	No. of measurements (n)	Linear equation $\hat{y}_i \text{ (mV)} = a \pm ts_a + b \pm ts_b [\text{Fe(II)}] \mu\text{g}.\text{ml}^{-1}$ at confidence level 95% , n - 2	r , r ² %	$t_{(0.05/2),11}$ at 95% confidence limit	$t_{\text{cal}} = \frac{ r \sqrt{11}}{\sqrt{1-r^2}}$
Iron ore 302	0-20	13	$\hat{y}_i \text{ (mV)} = 355.11 \pm 66.5 + 123.90 \pm 5.58 [x]$	0.9975, 99.50%	2.201 << 46.602	
Iron ore 303			$\hat{y}_i \text{ (mV)} = 188.35 \pm 78.51 + 63.81 \pm 7.62 [x]$	0.9849, 96.87%		

$\hat{Y}_i \text{ (mV)}$ = Estimated CL-response for (n=3), $[x] = [\text{Fe (II)}] \mu\text{g}.\text{ml}^{-1}$, r = correlation coefficient, r²% = linearity percentage. $t_{\text{tab}} = t_{0.05/2, n-2}$ at 95% confidence level

Table 10: iron (II) ion determination in two types of iron ore alloys (302,303) using Luminol_{G.B.}- H₂O₂-Fe(II) ion 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 taken sample alloy (g.)	[Fe(II)] ion $\mu\text{g}.\text{ml}^{-1}$	[Fe(II)] $\mu\text{g}.\text{ml}^{-1}$ after dilution 7.5ml/100 ml (Theoretically)	[Fe(II)] $\mu\text{g}.\text{ml}^{-1}$ (practical y)	Wt. of Fe in 100 g. sample (practical y)	Recovery %
302	0.0282	35.5	0.0100 theoretically	40.0	3.000	2.8666	33.92	95.56%
			9.555×10^{-3} practically					
303	0.0279	35.9	0.0100 theoretically			2.9517	35.32	98.49%
			9.849×10^{-3} practically					

CONCLUSION

A chemiluminescence – CFIA method is proposed for determination of Fe(II) ion and its applied successfully in determination of iron (II) ion in two types of iron ore alloy (302, 303, British chemical standards). The method based on oxidation of luminol (CL-donor) by hydrogen peroxide in the presence of Fe(II) ion as a catalyst metal ion for the CL- reaction. The proposed method is simple, rapid and sensitive.

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