INTERNATIONAL JOURNAL OF RESEARCH IN PHARMACY AND CHEMISTRY

Available online at www.ijrpc.com

Research Article

NOVEL CHEMILUMINO-FLUOROMETRIC METHOD FOR THE DETERMINATION OF Co(II) ION IN CoMo CATALYST TYPE (KF 124-3E) BASED ON THE IN SITU IRRADIATION OF RELEASED CHEMILUMINESCENCE FROM POLY ACRYLIC ACID GEL BEADS SATURATED WITH LUMINOL TO A FLUORESCEIN MOLECULE USING CONTINUES FLOW INJECTION ANALYSIS IN A SPECIALLY DESIGNED CELL

Issam MA Shakir[*] and Zaineb F Hassan

Department of chemistry, Collage of Science, University of Baghdad, Iraq.
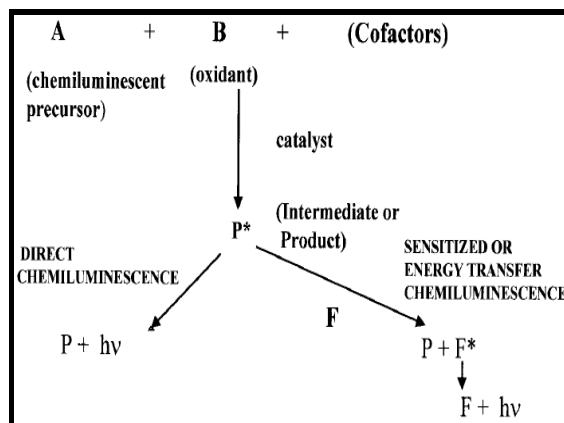
ABSTRACT
The study presents a sensitive, fast and easy assay method that was developed for determination of cobalt (II) ion via continuous mode of FIA i.e. total luminescence which include the chemiluminescence generated and the fluorescence that was created by the in situ radiation of the released chemiluminescence light. Using fluorescence molecule which gives an emission spectrum at 530 nm ifits subjected to a blue violet irradiates that's generated by a chemiluminescence well-known reaction system. The method is based on the absorbance of the donor molecule (Luminol) by fourteen poly acrylic acid gel beads located in specially designed cell, this cell will be the measuring cell instantly with the released Luminol from the gel beads. Experimental parameters that lead to optimum concentrations of all chemicals involved in the reaction was 5×10^{-5} M for Fluorescein and hydrogen peroxide and 1×10^{-3} M of Luminol. A sample volume of 40 μL was used throughout the whole work. It was found that the linear working range was between 0.08-7.0 μg.ml^{-1}, with correlation coefficient of 0.9789 and limit of detection L.O.D. (S/N =3) 120 pg/sample by using step wise dilution of the minimum concentration that was achieved by the calibration graph. Repeatability of less than 1.5% for five successive injections of 2.0μg.ml^{-1} of Co (II) ion solution. The method was applied successively in determination of Co (II) ion in CoMo catalyst type (KF 124-3E) that's used constantly in dehydrosulphurization processes (HD) of petroleum products.

Keywords: chemiluminescence, in situ fluorescence, CoMocatalyst (KF 124-3E), PAA gel beads.

1. INTRODUCTION
Cobalt is a fairly rare metal, comprising only 0.001 percent of earth's crust[1], its produced as a by-product of either nickel or copper[2]. Cobalt has been in use since at least 2250 BC. When it's used in coloring of jewelries, porcelain, glass, pottery, tile and enamels[3]. Catalysts containing cobalt, Molybdenum, nickel are extensively used in petrochemical processes, generally in hydro-desulphurization reactions (HD)[4] which are common to all refineries in the feed stock (crude oil fractions), and the aim is to convert the organic sulfur to H_2S since all crude oils contain sulfur in quantities ranging from 0.1-2.5% depending on their origins[5]. Several methods referring to the determination of Cobalt (II) ion demonstrating the importance of this element. Utilizing atomic absorption spectrometry[6-9], voltammetry[10, 11], inductively coupled plasma[12,13], UV-Vis[14-16] and chemiluminescence detectors[17-20]. Its well-known that chemiluminescence reactions are widely used

in analytical chemistry due to their high sensitivity, rapidity, simplicity and low instrumentation cost[21]. In general, two types of Chemiluminescence mechanisms can be distinguished in scheme 1[22].

Scheme. 1: types of Chemiluminescence reactions
F: fluorophore, *: excited state, hν: luminescence light

The expected mode of the realize of the in situ fluorescence is due to the irradiation of the photon released from the chemiluminescence emission which gives a maximum absorbance at 425 nm which is the same wave length of fluorescein molecule that absorbs this blue light to give the green band spectra at (530-532 nm), figure 1 and this was the basis of the measurement throughout this research work i.e. measurement of total luminescence[23,24].
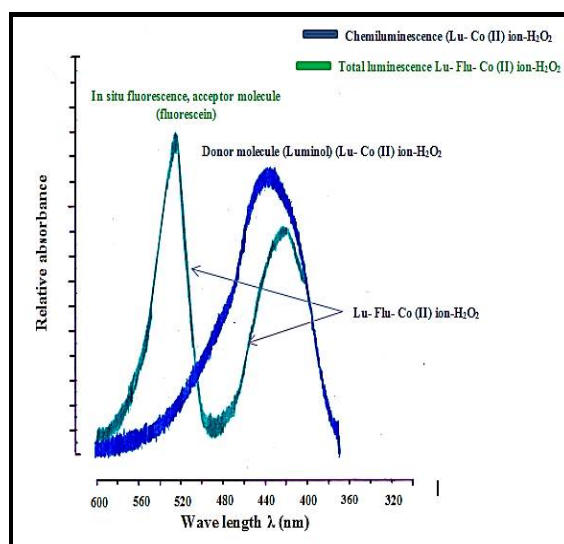

Fig. 1: Visible scanning of Chemiluminescence (Lu- Co (II) ion-H_2O_2)
& Total luminescence (Lu- Flu- Co (II) ion-H_2O_2) system

The main purpose of this work is to establish a simple, sensitive, and rapid chemiluminescence/fluorescent method for the determination of Cobalt (II) ion. This method combined with flow injection analysis technique and has been used for Co(II) ion determination in CoMo catalyst type (KF124-3E) with a satisfactory results.

2. Experimental
2.2. 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} Cobalt (II) ion as Co $(NO_3)_2.6H_2O$ (291.03 g.mol^{-1}, BDH) was prepared by dissolving 2.4691g in 500 ml distilled water. 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$_2$CO$_3$(105.97 g.mol^{-1}, BDH), prepared by dissolving 2.6493g in 500 ml distilled water. A stock solution of hydrogen peroxide H$_2$O$_2$ (1×10^{-2}mol.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$_2$C$_2$O$_4$ 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$_5$(332.31g.mol^{-1}, BDH) prepared by dissolving 0.3323g in 0.05 %NaOH then complete the volume with distilled water to 250 ml volumetric flask.

Preparation of Poly Acrylic Acid Gel Beads
Poly acrylic acid gel beads that haveweight ranges between 35.0-39.0.mgm, were washed and swelled in distilled water then dried using homemade drying cabinet.The dehydration process need about 132 hours at 45°C and relative humidity 8%. The sorted gel beads kept in clean and dry containers, sorting was based on their weight; which corresponds with its diameter due to its regular spherical shape. All these treatments of gel beads were made in order to obtain and in turn use regular unbiased sample of poly acrylic acid gel beads.

2.2. Preparation of Sample Solution (CoMo catalyst type KF124-3E)
A 100 ml solution of CoMo (K F124-3E) catalyst was prepared by dissolving 1.0 g of the Catalyst containing 0.04 % wt. of CoO in 25 ml of concentrated nitric acid (70%, 1.42g.mol^{-1}, BDH). This solution was heated on IR heater until the volume of acid reached to its half value, then a 50 ml of distilled water was added. Heating was continued until just about dryness. The rest of the remaining dissolution product was filtered through Whitman 595 filter paper to 100 ml volumetric flask. 0.8 ml of this dissolving product was transferred to each of a series of 100 ml volumetric flask which were prepared for standard addition curve.

2.3. 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).

2.4. Gel Bead Cell Unit (GBCU)
Figure 2 shows the photomultiplier tube (PMT) with the three dimensional arrangements and structural design of the GBCU.
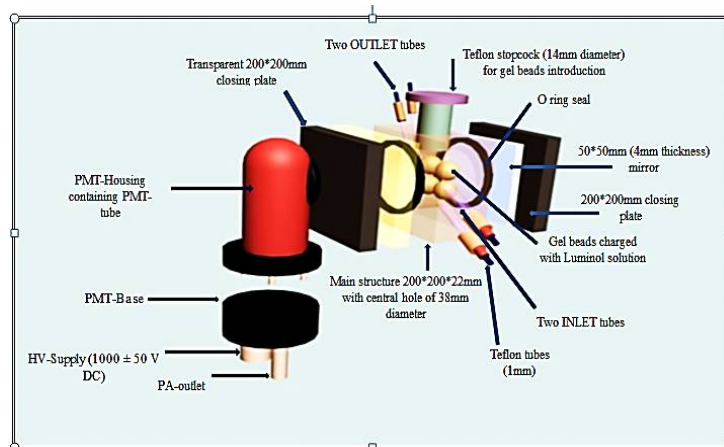

Fig. 2: three-dimensional structural design of the gel bead cell unit (GBCU)
** Random number of gel beads is used i.e. even lesser or more than four gel beads can bemanipulated in the GBCU

The cell is fitted with two inlet tubes since the cell is used for reaction, mixing and measuring purposes. Also two outlet tubes were used in order to avoid any retained air bubbles that might be trapped in the cell. A fourteen mm aperture is fitted for the introduction of poly acrylic acid gel beads. Cell body block is made from transparent poly methyl methacrylate of 200*200*22 mm with a hole of 38mm diameter. Two O-ring seals were used to prevent any leakage, two thin transparent 200*200 mm closing plates and two transparent protection plates; each one has four holes for the screws. Plain mirror of 50*50 mm and 4 mm thickness used on one side of the gel bead cell unit in order to insure the increase of light intensity falling on the photocathode of the photomultiplier tube results from multi-reflection process. The walls of the GBCU were fixed by four screws and nuts to ensure complete seal design, a group of silicone rubber tubes and connecting tubes of 1.5 mm used for connection of the manifold.

2.5. Methodology

Figure 3 shows the details of the manifold system used for the determination of Co(II) by the total luminescence of H_2O_2–Luminol$_{GB}$-Co(II) ion.
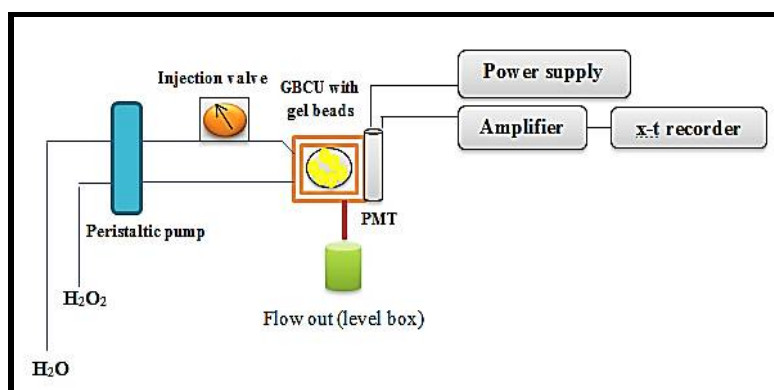

**Fig. 3: schematic diagram of the continues flow injection
analysis system with GBCU that used for determination of Co(II) ion**

Two line system was used, the first line using carrier stream of distilled water which passes through the injection valve carrying Co (II) ion sample segment to the measuring cell while the second line supplies hydrogen peroxide which is fed directly to the GBCU where a 14 gel beads are saturated previously by Luminol solution 1×10^{-3} M , this was carried out by passing Luminol solution in a close circuit from the peristaltic pump to the cell returning back the outlet to the main supplier beaker and this process can be repeated for many times if it was found that its necessary to refresh the gel beads with a new Luminol solution as its used as a donor molecule that will oxidized by hydrogen peroxide with aid of Co(II) ion to release the Chemiluminescence light that will be used to irradiate the fluorophore acceptor molecule.

A sample volume of 40μL and a flow rate of 2.0, 2.65 ml.min^{-1} for both carrier and hydrogen peroxide line respectively, using 50 seconds time to discharge the sample segment from the sample loop, this was established previously while working the Chemiluminescence method.The GBCU will work as a reaction-measuring cell equipped with the photomultiplier tube which is capable of measuring UV-Vis-IR region. Applied photocathode voltage of 600 VDC was used. Each injection is followed by a waiting time of three minutes which is found to be necessary for the refreshment of generated Luminol inside the GBCU. The output of the PMT is fed to dual detector (united detector technology, U.S.A.), the output of which is recorded on x-t potentiometric recorder (1-2 Voutput).Figure 4 shows three kinds of responses of the preliminary experiments.
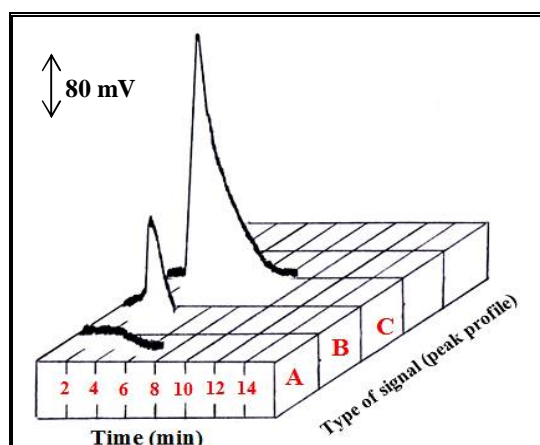
Fig. 4: peak- time profile of three successive signals, A: distilled water response, B: chemiluminescence response, C: total luminescence [**] **response– time profile.**
[**]**Total luminescence: Chemiluminescence plus in situ fluorescence by the Chemiluminescence irradiation of H_2O_2–Luminol$_{GB}$-Co(II) ion system**

3. RESULTS AND DISCUSSION
3.1. Optimization of FluorophoreMolecule Concentration (Fluorescein Sodium Salt)
Variable concentrations ofFluorescein sodium salt ranging from 1×10^{-5}- 5×10^{-4} M was mixed with a constant concentration of Co(II) ion (2.0 µg.ml^{-1}) in order to obtain the optimum concentration of fluorescein salt that gives the highest total luminescence emission. Table 1 sums up all the obtained results while figure (5- A, B) shows that 5×10^{-5} M of fluorescein salt is the optimum concentration.

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

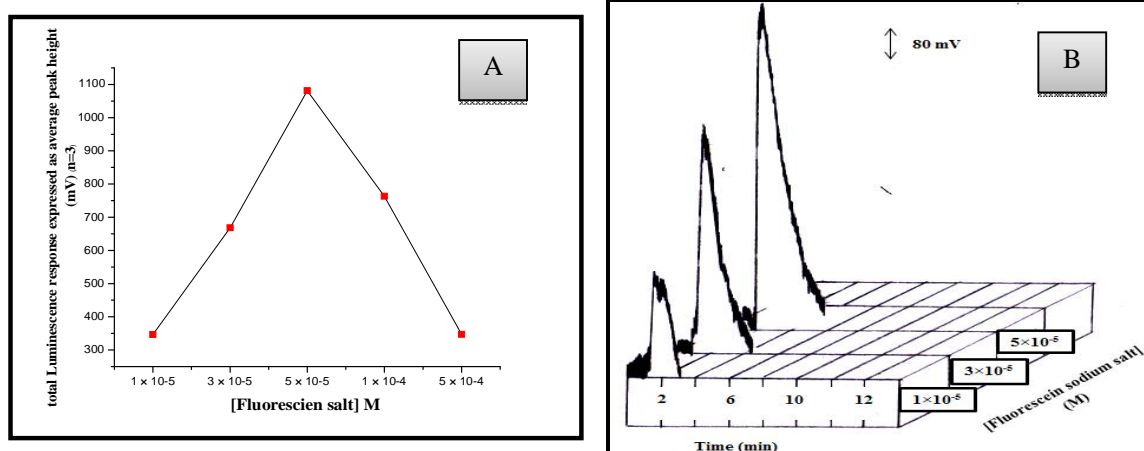| Conc. of Fluorescein salt (M)	Total luminescence responses expressed as peak height (mV)	Average ($\bar{y}_i$) (mV)	Standard deviation(SD) σ_{n-1}	Repeatability RSD%(SD/$\bar{y}_i$)*100%	Confidence interval of the mean at 95% limit $\bar{y}_i \pm t_{(0.05/2),n-1} \times (\sigma_{n-1}/\sqrt{n})$
1×10^{-5}	340, 348, 352	346.67	6.110	1.762	346.67 ± 15.181
3×10^{-5}	664, 667, 674	668.33	5.132	0.768	668.33 ± 12.750
5×10^{-5}	1080, 1084, 1079	1081	2.646	0.245	1081 ± 6.575
1×10^{-4}	760, 766, 763	763	3.000	0.393	763 ± 7.453
5×10^{-4}	352, 340, 349	347	6.245	1.800	347 ± 15.517

Fig. 5: A: variation of total Luminescence response versus Fluorescein sodium salt concentration.B: total Luminescence response-time peak profile for three different concentrations of Fluorescein sodium salt

3.2. Variation of Total Luminescence Response Versus Cobalt (II) Ion Concentration

A series of Co(II) ion solution prepared in 5×10^{-5} Mof Fluorescein sodium salt solution were prepared with concentrations ranging from 0.0-9.0µg.ml^{-1}. A linear calibration graph was found to have a correlation coefficient of 0.9789 for the range 0.08-7.0 µg.ml^{-1}. Details of the obtained results are tabulated in table 2.

Table 2: Variation of total luminescence response versus the concentration of Co (II) ion

[Co(II)] ion µg.ml^{-1}	Total luminescence 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 $\bar{y}_i \pm t_{(0.05/2),n-1} \times (\sigma_{n-1}/\sqrt{n})$
0.08	38, 41, 41	40	1.732	4.330	40 ± 4.299
0.1	75, 70, 71	72	2.646	3.675	72 ± 6.575
0.3	130, 134, 120	128	7.211	5.634	128 ± 17.913
0.5	134, 138, 136	136	2.000	1.471	136 ± 4.798
1.0	310, 308, 318	312	5.292	1.696	312 ± 13.146
3.0	641, 642, 640	640	1.000	0.156	640 ± 2.483
5.0	884, 889, 891	888	3.606	0.406	888 ± 8.959
7.0	1758, 1762, 1760	1760	2.000	0.114	1760 ± 4.970

The electronic system was adjusted in order to measure the upper limit signal of 7.0 and 9.0 µg.ml^{-1} otherwise, it will be out of scale and can't be measured i.e. the amplification through the project must be optimized to measure extended length of linearity (wide applicability) otherwise any PMT voltage can effect in an exponential form of responses. Figure 6 shows the variation of responses with Co(II) ion concentration, while Table 3 tabulates the statistical results of the calibration graph.
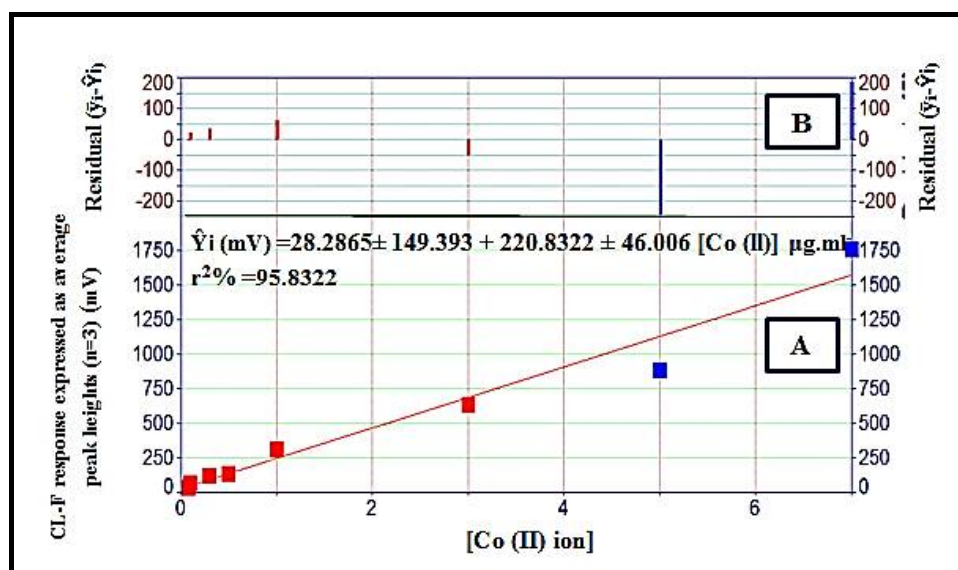

Fig. 6: Calibration graph for the variation of Co (II) concentration on:-
A: total luminescence response expressed by linear equation,
B:residual ($\bar{y}_i$– $\hat{Y}i$), $\bar{y}_i$: practical value, $\hat{Y}i$: estimated value

Table 3: Summary of calibration graph results for the determination of Co(II) ion by total luminescence of H_2O_2- Lu $_{GB}$- Co (II) ion system

Measured Conc. of Co(II)ion (µg.ml^{-1})	Linear dynamic range (µg.ml^{-1}) (n=8)	Linear equation $\hat{Y}i$ (mV) = a ± ts$_a$ +b ± ts$_b$ [x] µg.ml^{-1} at confidence level 95% , n- 2	r , r^2%	t$_{tab}$ at 95% confidence limit	t$_{cal}$ $t=\frac{|r|\sqrt{n-2}}{\sqrt{1-r^2}}$
0.0–9.00	0.08–7.00	28.2865± 149.393 + 220.8322 ± 46.006 [x] µg.ml^{-1}	0.9789, 95.8322%	2.447<< 11.7449	

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

3.3. Limit of detection (L.O.D)

A series of Cobalt (II) solutions in 5×10^{-5}M of fluorescein salt were prepared, the upper limit was 0.08 µg.ml^{-1} going down successively to distilled water which gives zero signal. Table 4 shows L.O.D. conducted by three different methods.

Table 4: Summary of limit of detection based on different approaches at 40 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 $\hat{Y}i \ (mV) = Y_B + 3S_B$
120 pg/sample	1.772 ng/sample	71.00 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)

3.4. Repeatability

Repeatability of measurements was studied at two variable concentration of Cobalt (II) solutions in 5×10^{-5} M of fluorescein salt namely, 2.0, 5.0 µg.ml^{-1} at optimum parameters. The repeated measurements for five successive injections were measured and tabulated in table 5, while figure (7-A, B) shows a kind of response-time profile for the used concentrations.

Table 5: Repeatability results of Co(II) ion at optimum parameters by CFIA-CL/fluorescence method

[Co(II) ion] µg.ml^{-1}	No. of injections	Total luminescence response expressed as average peak height (mV)	Standard deviation σ_{n-1}	RSD %	Confidence interval of the mean at 95% $\bar{y}_i \pm t_{0.05/2, \ n-1} \times \sigma_{n-1} / \sqrt{n}$
2	5	396.6	5.273	1.330	396.6 ± 6.546
5	5	839.0	7.874	0.938	838.4 ± 9.774

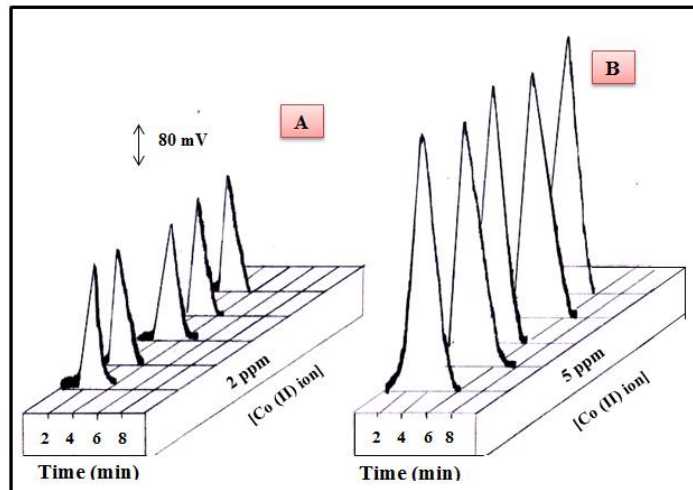**Fig. 7: The total luminescence response-time profile for five successive repeatable measurements of Co(II) ion; A: 2.0 µg.ml^{-1}, B: 5.0 µg.ml^{-1}**

Applications

The established method was used for the determination of cobalt (II) ion in CoMo catalyst (KF 124-3E AXSENCE-France). The standard addition method was applied by preparing a series of solutions via transferring 0.8 ml of catalyst dissolution product to each of eight volumetric flasks (100 ml), followed by the addition of (0.0, 1.5, 2.5, 3.0, 3.5, 4.0, 6.0 and 7.0 ml) from the standard solution of Co(II) ion (100 µg. ml^{-1}) prepared in 5×10^{-5} M of fluorescein salt solution, in order to have the concentration range from (0.0-7.0µg. ml^{-1}) for the preparation of standard addition calibration plot. 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
Co(II) ion by in situ fluorescence from Luminol$_{G.B.}$-H$_2$O$_2$-Co(II) ion (standard addition method)**

Measured Conc. of Co(II) ion (μg.ml^{-1})	No. of measure-ments (n)	Linear equation $\hat{Y}_i$ (mV) = a $\pm$ ts$_a$ +b $\pm$ ts$_b$ [x] μg.ml^{-1} at confidence level 95% , n- 2	r , r^2%	t$_{tab}$ at 95% confidence limit	t$_{cal}$ $t=\dfrac{\left\|r\right\|\sqrt{n-2}}{\sqrt{1-r^2}}$
0.0–7.00	8.0	150.2537$\pm$ 95.399+77.3080$\pm$23.599 [x]	0.9563, 91.46	2.447 < 7.800	

$\hat{Y}_i$ (mV) =Estimated CL-F response for (n=3), [x] = [Co (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: Co(II) ion determination in CoMo catalyst type (K F124-3E) by in situ fluorescence
from Luminol$_{G.B.}$-H$_2$O$_2$-Co(II) ion by standard addition method**

Type of sample	Wt. of sample (g.)	Wt. of CoO in sample (g.)	Wt. of Co(II) ion in sample (g.)	[Co(II)] μg.ml^{-1}	[Co(II)] μg.ml^{-1} after dilution 0.8 ml/100 ml (Theoretically)	Average of CL-response (n=3), (mV)	[Co(II)] μg.ml^{-1} (practically)	Recovery %
CoMo catalyst (K F124-3E)	1.00	0.04	0.03146	247.4632	2.000	184.00	1.944	98.20%

3.6. CONCLUSION

The present paper introduce a simple and sensitive approach for the determination of Co(II) ion via total Luminescence i.e. chemiluminescence plus the in situ fluorescence by the Chemiluminescence irradiation of Luminol$_{G.B.}$- H$_2$O$_2$-Co(II) ion system. An improved linearity and detection limit compared with the available literatures cited in the introduction. The new method was used successfully for determination of Co(II) ion in CoMo catalyst (KF 124-3E AXSENCE-France) without any need for pretreatments.

REFERENCES

1. Enghag P. Encyclopedia of the elements; Technical data-History- Processing-Applications, Wiley-VCH, New York, 2004;667.
2. Hawkins MJ. Recovering cobalt from primary and secondary sources. Journal of Minerals, Metals and Materials Society.1998;50(10):46-50.
3. Earnshaw A and Norman Greenwood. Chemistry of the Elements, 1997,2nd Edition, Elsevier Butterworth Heinemann, London, 1117-1119.
4. Barika. SP, Parka KH, Parhia PK, Parka JT and Nama CW. Extraction of metal values from waste spent petroleum catalyst using acidic solutions. Separation and Purification Technology. 2012;101:85–90.
5. Yasuaki Okamoto. Preparation and application of Co-Mo model sulfide catalysts for hydrodesulfurization. Prepr. Pap Am Chem Soc Div Fuel Chem. 2003;248(2):599-600.
6. HosseinAbdolmohammad-Zadeh and ElnazEbrahimzadeh. Determination of cobalt in water samples by atomic absorption spectrometry after pre-concentration with a simple ionic liquid-based dispersive liquid-liquid micro-extraction methodology.Central European Journal of Chemistry. 2010;8:617-625.
7. Neda Baghban, Ali Mohammad H, Shabani, Shayessteh Dadfarnia and Abbas A Jafari. Flame atomic absorption spectrometric determination of trace amounts of cobalt. J Braz Chem Soc. 2009;20(5):832-838
8. Paula Bertona and Rodolfo G Wuilloud. An online ionic liquid-based microextraction system coupled to electrothermal atomic absorption spectrometry for cobalt determination in environmental samples and pharmaceutical formulations. Anal Methods. 2011;3:664-672.
9. Jianrong Chen and Khay Chuan Teo. Determination of cobalt and nickel in water samples by flame atomic absorption spectrometry after cloud point extraction. Analytica Chimica Acta. 2001;434(2):325–330.
10. Othman A Farghaly. Direct and simultaneous voltammetric analysis of heavy metals in tap water samples at Assiut city: an approach to improve the analysis time for nickel and cobalt determination at mercury film electrode. Microchemical Journal. 2003;75(2):119-131.
11. Marisol Vega, Constant MG and van den Berg. Determination of Cobalt in Seawater by Catalytic Adsorptive Cathodic Stripping Voltammetry. Anal Chem. 1997;69(5):874-881.
12. SreenivasaRaob K, Balajib T, PrasadaRaoc T, Babud Y and Naidua GRK. Determination of iron, cobalt, nickel, manganese, zinc, copper, cadmium and lead in human hair by inductively

coupled plasma-atomic emission spectrometry. Spectrochimica Acta Part B. Atomic Spectroscopy. 2002;57(8):1333–1338.

13. NédioOleszczuka. Jacira T. Castrob.Márcia M. da Silvaa. Maria das Graças A. Kornb. Bernhard Welzb. Maria Goreti R. Valea. Method development for the determination of manganese, cobalt and copper in green coffee comparing direct solid sampling electrothermal atomic absorption spectrometry and inductively coupled plasma optical emission spectrometry. Talanta. 2007;73 (5): 862–869.

14. Issam MA, Shakir Nagam and Turky S. Spectrophotometric-contiguous flow injection analysis (S-CFIA) for the determination of cobalt (II) ion in river & alloy samples via the use of 10,10-dimethyl 9,9-diacridinium dinitrate salt hydrogen peroxide in alkaline medium. Journal of Karbala University. 2013;11(3):244-258.

15. Safavia A, Abdollahib H, HormoziNezhada MR and Kamalia R. Cloud point extraction, preconcentration and simultaneous spectrophotometric determination of nickel and cobalt in water samples. SpectrochimicaActa Part A: Molecular and Biomolecular Spectroscopy. 2004;60(12):2897–2901.

16. Jamaluddin Ahmed M and Nasir Uddin M. A simple spectrophotometric method for the determination of cobalt in industrial, environmental, biological and soil samples using bis(salicylaldehyde)orthophenylenediamine.Chemosphere. 2007;67(10):2020–2027.

17. Edward A Boyle, Brent Handy and Alexander Van Geen. Cobalt determination in natural waters using cation-exchange liquid chromatography with luminolchemiluminescence detection. Anal Chem.1987;59(11):1499–1503.

18. Shinjiro Nakahara. Masaaki Yamada. Shigetaka Suzuki. Chemiluminescence for the determination of traces of cobalt(II) by continuous flow and flow injection methods. Analytica Chimica Acta.1982;141:255–262.

19. Carole M, Sakamoto-Arnold and Kenneth S Johnson. Determination of picomolar levels of cobalt in seawater by flow injection analysis with chemiluminescence detection. Anal Chem.1987;59(14):1789–1794.

20. Masaaki Yamada. Takayuki Komatsu. Shinjiro Nakahara1. Shigetaka Suzuki. Improved chemiluminescence determination of traces of cobalt (II) by continuous flow and flow injection method. AnalyticaC himica Acta.1983;155: 259–262.

21. George Z, Tsogas, Dimitrios V, Stergiou, Athanasios G, Vlessidis, Nicholaos P and Evmiridis. Development of a sensitive flow injection-chemiluminescence detection method for the indirect determination of propranolol. AnalyticaChimicaActa. 2005;541(1–2): 149–155.

22. Issam MA, AL-Hashimi, Nagam ST and AL-Awadi. New developed analytical-chemiluminometric (sensitized) continuous flow injection analysis for determination of ciprofloxacine; application to pharmaceutical preparations. Journal of AL-Nahrain University. 2012;15(4):11-21.

23. García-Campaña AM and Baeyens WRG. Chemiluminescence in Analytical Chemistry, Marcel Dekker, Inc. New York, 2001.

24. Podemska K, Orzeł A, Podsiadły R and Sokołowska J. Chemiluminescence-mystery of cold light. Chemik. 2013;11:1091-1096.