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New Mode of On- Line Automation: Chemilumino-Fluoro Metric Method for the Determination of Hydrogen Peroxide by Fluorescence Energy Transfer Using Ploy Acrylic Acid Gel Beads

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Abstract

Hydrogen peroxide was determined by a new , accurate , sensitive and rapid method via continuous mode of FIA coupled with total luminescence measurement which include the chemiluminescence generated ,based on the oxidation of Luminol which is loaded on poly acrylic acid gel beads by hydrogen peroxide in presence of Cobalt (II) ion as a chemiluminescence catalyst and the fluorescence that was created by the insitu radiation of the released chemiluminescence light. Fluorescien molecule was used as an accepter fluorophore where it is irradiated internally and instantly by the generation of luminol chemiluminescence light as internal source for irradiation of fluorescien molecule (Fluorescence Energy Transfer (FRET)) . It can easily give fluorescence light (i.e , no external source for irradiation is used) at λ_{max} =530 nm. 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 measure instantly the emitted light (FRET) . A sample volume of 80 µL was used throughout the whole work. Linear calibration curve extend from 0.1 - 100 µMol $.L^{-1}$, with correlation coefficient of 0.9985 and limit of detection L.O.D (S/N =3) 217.60 pg/sample using step wise dilution of the minimum concentration that was achieved by the calibration graph. Repeatability (RSD%) of less than 0.5% for six successive measurement of 50 µMol $.L^{-1}$ of hydrogen peroxide. The method was applied successively in determination of hydrogen peroxide in some pharmaceutical disinfectants .

Keywords: Chemiluminescence, Flow injection analysis, gel beads, hydrogen peroxide, in situ fluorescence.

نمط جديد أني شبه تلقائي بطريقة البريق الكيميائي والفلورة لتقدير بيروكسيد الهيدروجين بوساطة الفلورة بأنتقال الطاقة بأستخدام حبيبات الجل لمتعدد حامض الاكريلك

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الخلاصة :

قدر بيروكسيد الهيدروجين بطريقة جديدة ومضبوطة وحساسة وسريعة عن طريق نمط التحليل بالحقن الجرياني المستمر والمزدوج مع قياس التألق الكلي والذي يتضمن توليد البريق الكيميائي بالاستناد على اكسدة اللومينال المحمل على حبيبات الجل لمتعدد حامض الاكريلك بوساطة بيروكسيد الهيدروجين بوجود ايون الكوبلت (II) كعامل مساعد للبريق الكيميائي ، اما الفلورة والتي تنشأ بوساطة التشعيع الأني لضوء البريق

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الكيميائي المتحرر بأستخدام الفلورسين كجزيئة فلوروفورية والتي بدورها تشعع داخلياً وأنياً من ضوء البريق الكيميائي للومينال والمستخدم كمصدر داخلي لتشعيع جزيئة الفلورسين . لذلك بالأمكان اعطاء ضوء الفلورة (يعنى ذلك لايوجد مصدر داخلي للتشعيع) عند 530 نانومتر . تستند الطريقة على امتصاص الجزيئة الواهبة (اللومينال) بوساطة 14 حبة جل من حامض متعدد الاكريلك والمحتواة في خلية بتصميم خاص والتي تمثل خلية القياس الأني بعد تحرير اللومينال من حبيبات الجل . 80 مايكرولتر حجم مقطع الانموذج المستخدم خلال العمل الحالي . يمتد منحني المعايرة من 0.1 – 100 مايكرولتر . لتر$^{-1}$ بمعامل ارتباط 0.9985 وحدود كشف (S/N=3) 217.60 بكغم / انموذج بأستخدام التخفيف التدريجي لأقل تركيز في منحني المعايرة . التكرارية اقل من 0.5 % لست حقنات متتالية لتركيز 50 مايكرومول . لتر $^{-1}$. طبقت الطريقة بنجاح في تقدير بيروكسيد الهيدروجين في بعض المطهرات الصيدلانية .

Introduction

Hydrogen peroxide is a clear, colorless liquid with a characteristic odor that is always present in aqueous solution at different concentrations. At high concentrations, hydrogen peroxide causes irritation of the eyes and skin and affects human health [1- 3]. Hydrogen peroxide plays a significant role in the chemical, pharmaceutical, industries and corrosion [4-6], Further, the detection of hydrogen peroxide is an important task in many biological, medical, electrochemical sensing and clinical studies [7-10] . Hydrogen peroxide is used as oxidant, disinfectant and bleaching agent in various industrial and household applications [11]. Literature survey shows that various analytical methods have been reported for the determination of hydrogen peroxide due to the fact that hydrogen peroxide plays significant role in many fields including food, pharmaceutical, chemical, biochemical industries, clinical control and environmental protection through its oxidation effect which generate several active oxygen species , superoxide ,hydroxyl , radicals or singlet oxygen ,etc. via different chemiluminescence systems with low injection asalysis [12-15].

Chemiluminescence reaction of Co(II)-H_2O_2 -Luminol is one of the most well known reaction which is catalysed by different metal ions [16-19] . Total luminescence measurement was first used by Shawkat [20] and Yousif [21] which is based on the idea of conducting a chemiluminescence reaction and transfer part of the energy to irradiate a fluorescence molecule, which in turn releases a fluorescence light for determination of H_2O_2 [22], thus increase the amount of photon flux released from both modes of reactions .These kinds of reaction necessitate the use of a modified mode of mixing and conducting of the reaction with a specific speed to avoid any losses of photon energy before detection point i.e; reaction and detection must be done instantly (insitu) infront of the detection unit via multi-detection points through optical fibers [23] in a completely newly designed cell [24] .Many fluorophore molecule was used to determine H_2O_2 such as Rhodamine -6G [25] and Rhodamine-B [26] by total luminescence (chemiluminescence and fluorescence) .

In this method ,the use of pre- absorbed luminol in poly acrylic acid gel beads that serve as a reserviour for the donor molecule to initiate a source of insitu irradiation for a fluorescent molecule to form a mixed and combind spectral light of remained chemiluminescence light and an insitu induced a fluorescent emission figure. 1 [23]. Both lights are generated inside a specially designed cell. The emitted total light was used as a new methodology for the determination of hydrogen peroxide and its application.

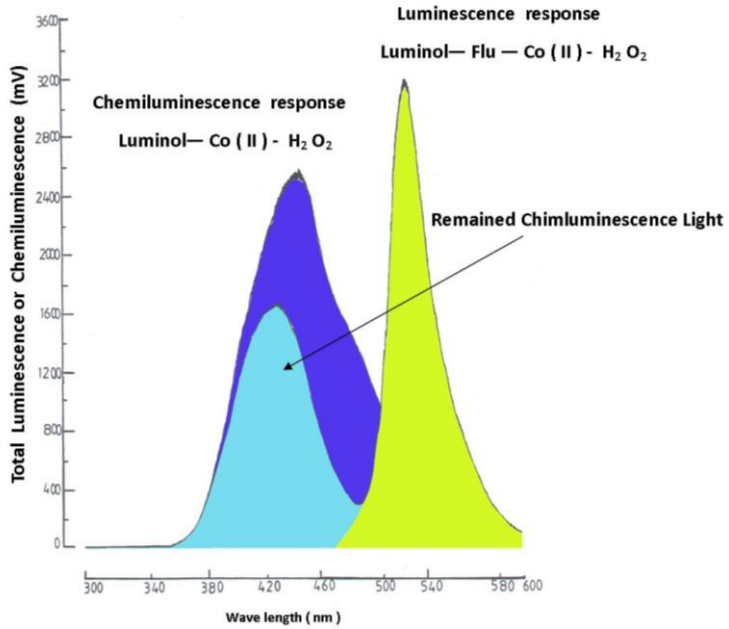


Figure 1-Visible scanning of Chemiluminescence (Luminol - Co (II) ion-H_2O_2) and Total luminescence (Luminol -Flu- Co (II) ion-H_2O_2) system ,using czerney-turner monochrometer.

Experimental

Chemicals and reagents

All used chemicals were 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 4.93830g in 1L distilled water. A stock solution (1mMol.L^{-1}) of Luminol solution (5-amino phthalylhydrazide) $C_8H_7N_3O_2$ (177.16 g.mol^{-1},BDH) was prepared by dissolving 0.17716g in 1L of 0.1mol.L^{-1} solution of sodium carbonate Na_2CO_3(105.97 g.mol^{-1} , BDH), prepared by dissolving 21.198g in 2L distilled water. A stock solution of hydrogen peroxide H_2O_2 (100mMol.L^{-1}) was prepared by pipetting 19.44 ml of hydrogen peroxide (35%, 34.01 g.mol^{-1}, 1.01g. ml$^{-1,}$ Fluka.) and complete the volume with distilled water to 2L volumetric flask. Hydrogen peroxide molarity was fixed in sulfuric acid medium (1:1) with potassium permanganate solution $KMnO_4$ (0.1 mol.L^{-1}) (158.03g.mol^{-1}, HopkinandWilliam) 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 (1mMol.L^{-1}) of fluorescein sodium salt $C_{20}H_{10}O_5$ Na_2(376.27g.mol^{-1}, BDH) prepared by dissolving 0.37627g in 0.1mol.L^{-1} solution of sodium carbonate then complete the volume to 1L volumetric flask.

Preparation of poly acrylic acid gel beads [27].

Poly acrylic acid gel beads that have weight ranges between 35.0-39.0.mg, were washed and swelled in distilled water then dried using homemade drying cabinet figure.2. The dehydration process need about 132 hours at 45^{0}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.

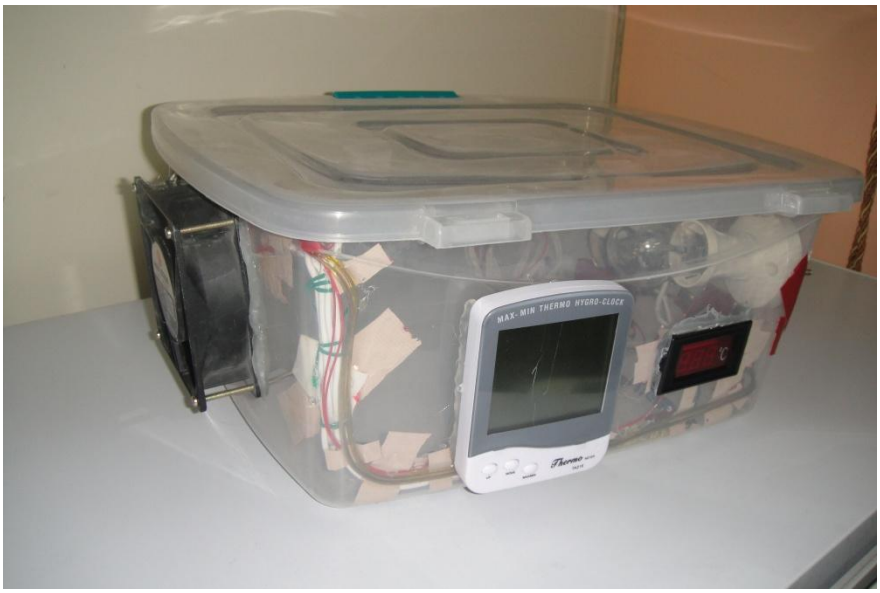


Figure 2-Gel bead evaporation unit

Sample preparation

Analysis of H_2O_2 in three different of pharmaceutical preparation (Baghdad company, Al-Amire company and Al- Areje company) have 25% (7.35 mol L^{-1}) , 20% (5.88 mol L^{-1}) and 7% (2.06 mol L^{-1}) concentration respectively **.** After standardization with $KMnO_4$ solution, the concentration of those samples obtained as 9.45%, 1.74% and 1.24% respectively**,** and a series of solutions 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 (1mV-500 V) (KOMPENSO GRAPH C-1032) SIEMENS (Germany).

Gel bead cell unit (GBCU):

The cell is composed of two chambers (an internal and an outsider) .The gel beads are located in the inner chamber (as a storage for luminol) . The wall between the two chamber has eight holes figure.3-A from which luminol can pass from the inner chamber through the holes to the reaction area where it meet the complementary reactant to generate the total luminescence (remained chemiluminescence and fluorescence)based on the inside irradiation of released chemiluminescence light (λ_{max} 425 nm) which serve as a source to stimulate instantly fluorescence (fluorescence energy transfer (FRET) .Measuring cell has two inlets and one outlet .

Figure.3- B shows O-Rings of two different diameters to entrap both solution and beads. In addition, a reflecting mirror at the backside to increase intensity of released luminescence. A well protection from scattered and outside light was established to have zero darkness.

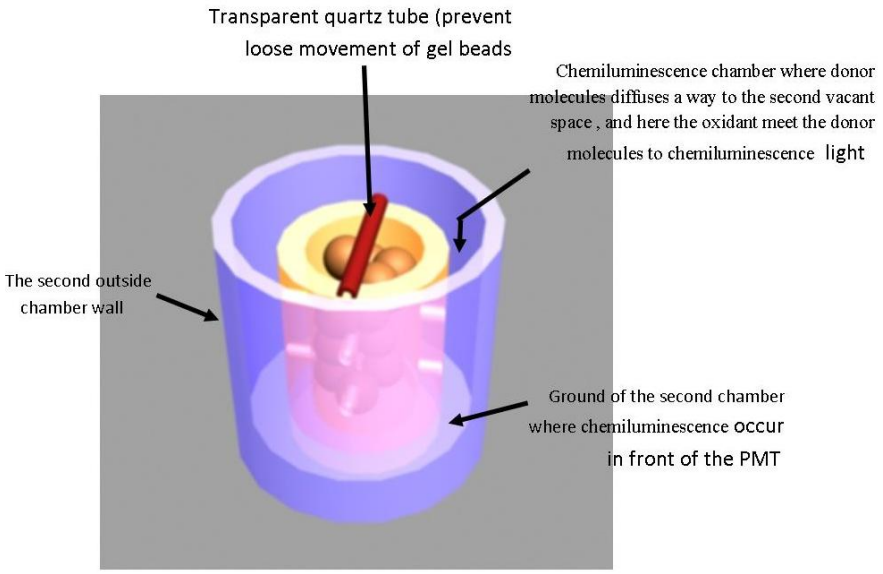


Figure 3-A-3D- representation showing the location of the first chamber , and the second one , also it shows the gel beads (water crystals) showing the glass tube (pink colored) , it is function is to : 1- prevention free movements 2- facilitate the formation of unblocked passage when gel bead expand (in addition to the eight available holes)

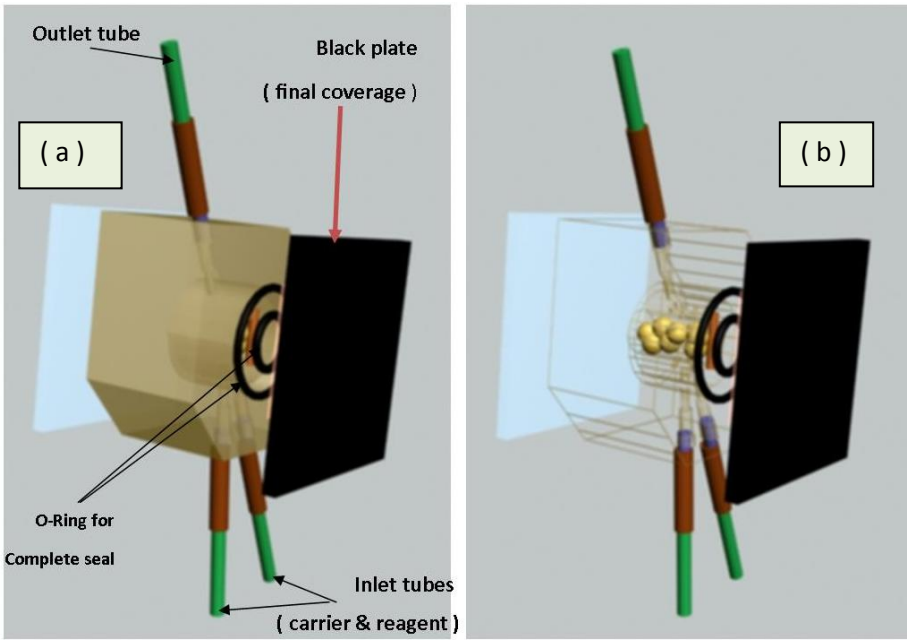


Figure 3-B-a-Circumferential Chemiluminescence cell in its final form
b -3D- net hidden structure for Gel bead cell unit

Methodology:

The manifold unit that was used for the determination of hydrogen peroxide as shown in figure. 4 by total luminescence: $Luminol_{GB}$-Flu -Co (II) ion- H_2O_2 system , which was composed of two lines , the first line supplies distilled water as a carrier stream at flow rate of 1.5ml.min^{-1} which leads to the injection valve to carry the segment of a mixture hydrogen peroxide and fluorescien solution at 80 µLsample volume ; while the second line supplies Co (II) ion at 5 µ g.ml^{-1} at flow rate of 2ml.min^{-1} . These two lines met and mixed at the GBCU where 14 gel beads which will supply the donor molecule i.e. Luminol (0.5mMol.L$^{-1)}$ that is necessary for the completion of the CL-reaction. Luminol will be released by the action diffusion figure 5 from within the 14 gel beads to the surrounding environment in which Cobalt (II) ion, fluorescien and Hydrogen peroxide will mix and the process of Chemiluminescence and fluorescien will take place. Scheme 1 shows the mechanism of Luminal

oxidation by hydrogen peroxide in presence of Co (ll) ion as a catalyst to release the Chemiluminescence light that will be used to irradiate the fluorophore acceptor molecule.

Two inlets were used (care was take due to the expansion of the gel beads). The duration of injection period was 40 seconds for complete sample discharge. 2.5 minutes as a time lag period was left between each two successive measurements. The obtained CL-response was recorded via photomultiplier tube (PMT) and on x-t potentiometric chart recorder.

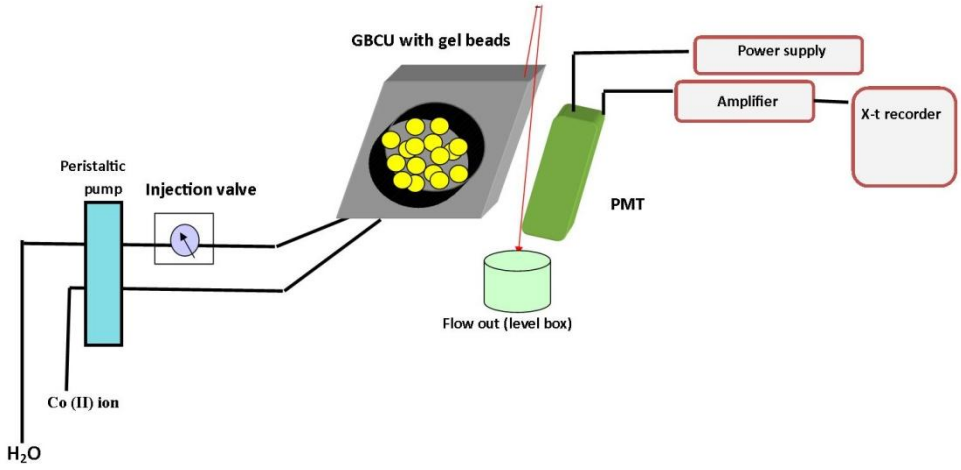
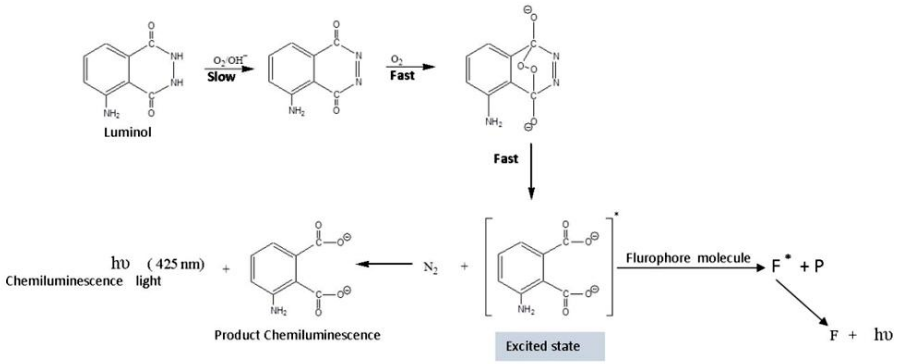


Figure 4-Schematic diagram of the continues flow injection analysis system with GBCU that used for determination of H_2O_2 by total luminescence



Scheme 1- proposed mechanism of $Luminol_{GB}$ – Flu - Co (II) ion - H_2O_2 system to measure total luminescence for determination of H_2O_2.

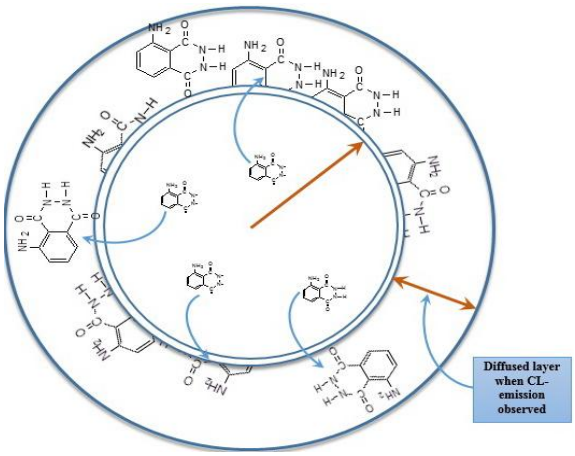


Figure 5-The probable mechanism of Luminol departure from gel bead via diffusion

Results and discussion:

Optimization of variables

Chemical as well as physical parameters were studied by employing the flow injection manifold that was shown in figure.1, in order to obtain the optimum parameters for the conditions of the chemiluminescence reaction.

Chemical variables:

Luminol concentration and gel bead numbers:

Figure. 6-A, B shows that at low luminol concentration which is the main source for the excitation of fluorescence molecule (Uranine) gives low fluorescence intensity which is expected to be so as the fluorescence intensity will relay on the intensity of the source (in this case it is insitu irradiation of fluorescence molecule by the generated photons from the donor molecule i.e. luminol).At higher luminol concentration with limited amount of available fluorescence ; not enough fluorescien molecules are available to give fluorescence in addition to duration of Chemiluminescence light is very short compared to fluorescence;0.6 sec for Chemiluminescenc and 45 min for fluorescence [28] .Inner filter effect could be added as a fluorescence is effected by this .

On this basis, A detailed preliminary work indicated that most probable useful for the purpose of determination is the use of 14 gel beads as it helps in an increasing the capability of being a good reserviour for luminol at 0.5 mMol.L^{-1} . It also give an increased surface area i.e. increased luminous area while reaction is conducted as the increase of surface area will spread the generated photon. This will ensure the avoidance or a decreased effect of inner filter effect. Also it gives more room for the energy transfer from the emitted Chemiluminescence light to a fluorescence molecules. This is the most important aim of the research work.

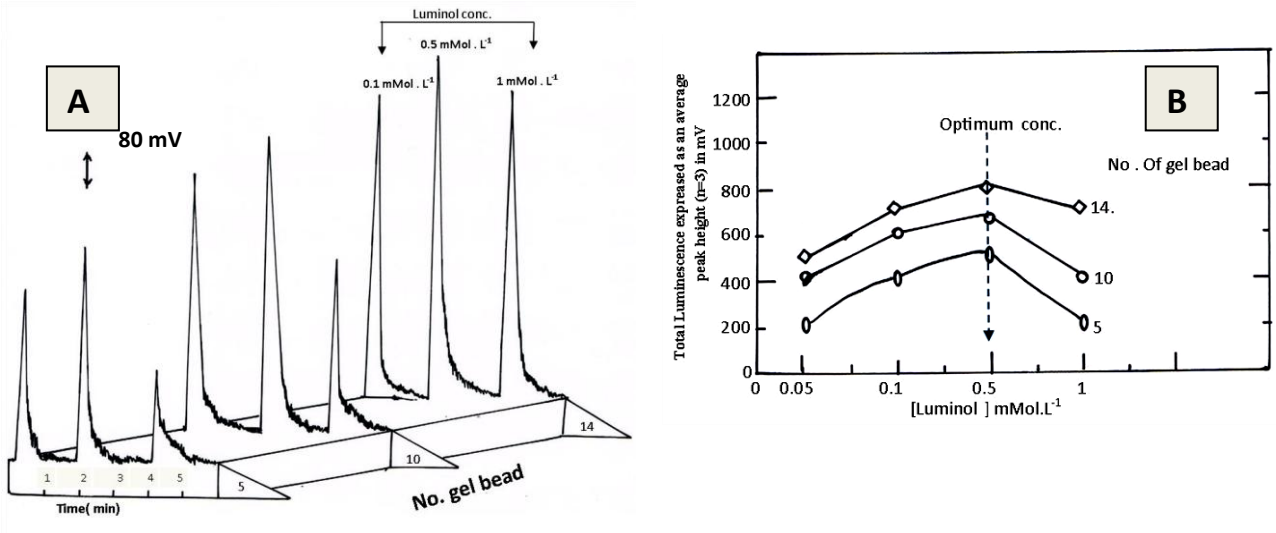


Figure. 6-A: variation of total Luminescence response versus Luminol concentration and no of gel bead,
B: Effect of Luminol concentration on the in situ fluorescence emission expressed as an average peak heights in (mV) using 70 µMol .L^{-1} H_2O_2 ,50µl , 3 µg.ml^{-1} Co(II) and [fluorescien] = 50 µMol .L^{-1} mixed with H_2O_2 ,as an injected sample.

Effect of either Co (II) ion and fluorescien concentration on total Luminescence:

The use of variable concentration of either Co (II) ion as a catalyst (3,5,7,10) µg.ml^{-1} and fluorescien (10,50,100,500,1000) µMol .L^{-1} mixed with a constant concentration of Hydrogen peroxide (70 µMol .L^{-1}) was used as an oxidant for Luminol to generate the CL-emission as a sample loop (50µl) , using open valve mode at a flow rate of carrier and reagent stream 1.3ml.min^{-1} and 1.8 ml.min^{-1} respectively. A study was carried out to optimize the concentration of Co (II) ion and fluorescien that will be used for the rest of this work. Figure7-A shows the response profile of emission versus concentration , while Figure7-B show the effect of either Co (II) ion and fluorescien concentration on total Luminescence .It indicate that at high concentration of Co (II) ion lead to a constant emission therefore 5 µg.ml^{-1} most suitable due to unnecessary consumption of high reagent

concentration even though that minimum concentration of fluorescien was 100 µMol .L^{-1} is the best to obtain suitable sensitivity .Any increase in fluorescien concentration causes a deformed response and decrease of response height might be due to the self quenching or due to the saturation of electronic system i.e. a constant saturated signals obtained.

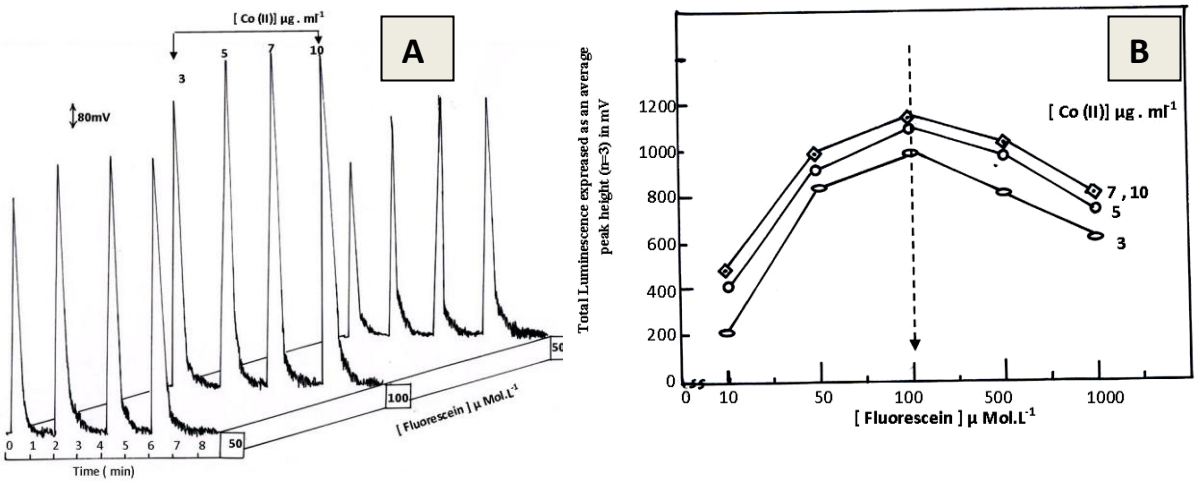


Figure 7-A: total Luminescence response- time peak profile for three different concentrations of Fluorescein sodium salt and four different concentrations of Co (II) ion .

B: variation of total Luminescence expressed as an average peak heights (mV) versus Fluorescein sodium salt concentration and Co (II) ion

Physical variable

Effect of flow rate and sample volume

Using optimum concentration of the reactant: Co (II) ion 5 µg.ml^{-1} and fluorescien conc. 100 µMol .L^{-1} mixed with a constant concentration of Hydrogen peroxide (70 µMol .L^{-1})for the optimization of flow rate that ranged from 0.4 – 3.1 ml.min^{-1} for Co (II) ion, and 0.4 – 2.7 ml.min^{-1} for the carrier stream. In addition to variable sample volume (50 , 80 , 100) µl with open valve technique. Figure 8-A shows the effect of flow rate on the total Luminescence(Chemiluminescence plus in situ fluorescence by the Chemiluminescence irradiation of H_2O_2 –$Luminol_{GB}$-Co (II) ion system) response- time peak profile. It was noticed that at low flow rates, there were an increase in peak base width (Δt_b).This might be due to the dispersion which causes an irregular response . While at higher flow rate (> 1.5, 2 ml.min^{-1} for the carrier stream and Co (II) ion respectively), although the effect of physical parameter was very crucial on the response profile; leading to regular response and very sharp maxima

Figure 8-B shows that the best flow rate for the completion of the reaction of Hydrogen peroxide as an oxidant for Luminol to generate the CL-emission at the presence of Co (II) ion as a catalyst for irradiation of the photon to the fluorescein molecule was 1.5 , 2 ml.min^{-1} for the carrier stream and Co (II) ion respectively to obtain a regular response , narrower Δt_b ,and minimize the consumption of reactants solutions . The time required from the moment of departure of the sample from the injection valve to the GBCU is 18 sec.

At the same time , It was noticed that an increase in sample volume led to an increase in the height of response profile for total Luminescence without affecting on the response profile of obtained signal up to the sample volume 80 µL. Above 80 µL, there were a broadening at the peak maxima and an increase in the base width (Δt_b) this is illustrated in figure 8-A which shows that the optimum volume was 80 µL for better response profile.

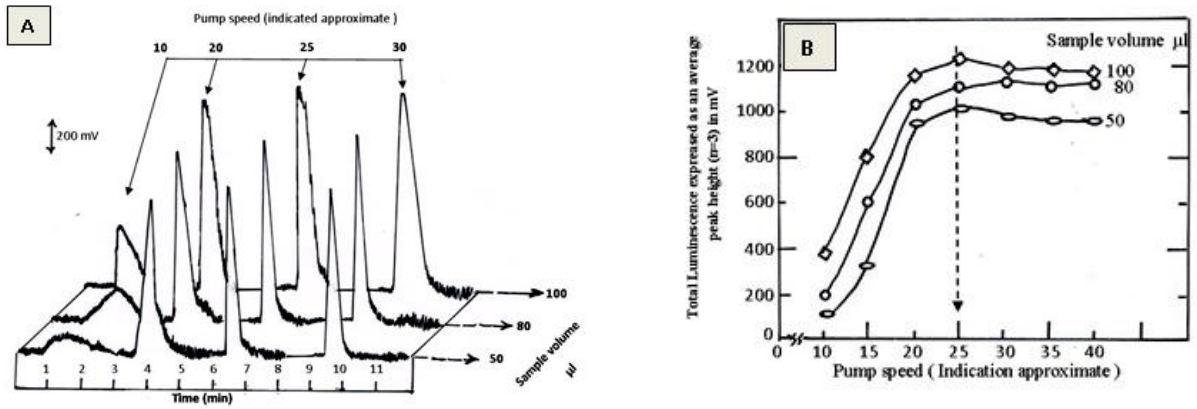


Figure 8-Effect of variation of the flow rate on

A- Total Luminescence response- time peak profile

B- B- Total Luminescence expressed as an average peak heights (mV).

Reactivation time for gel bead

A study was conducted to fix the optimum recharging time at stop-go mode of operation. The allowed recharging time for the absorption of Luminol then to re-supply the reaction medium with the CL- donor molecule from within the gel beads (diffused Luminol solution). Using the optimum parameters that were achieved in previous sections, a time laps of recharging period ranged from 0-3 minutes was used for the cell to be ready for next measurements .

Figure 9 (A,B) shows that in general an increase in recharging time (waiting period till the next injection) gives a higher total Luminescence.

The most suitable time that is necessary to supply enough luminol concentration for the reaction which is equivalent in term of time unite of 2.5 min while more than this ; will cause excess luminol that will give a high intensity reaching saturation level of the detector ; Therefore; a waiting period of 2.5 minutes was adopted as an optimum reactivating time.

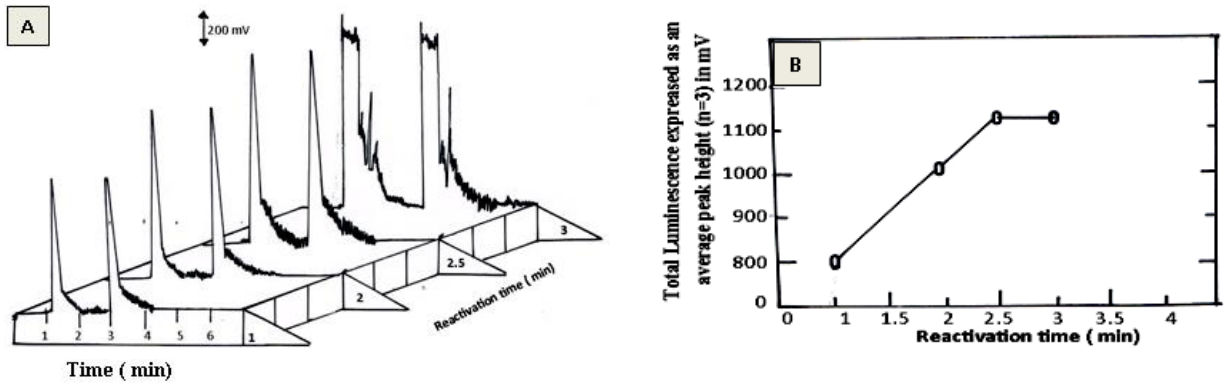


Figure. 9-Effect of reactivation time on

A- Total Luminescence response- time peak profile.

B- B- Total Luminescence expressed as an average peak heights (n=3) in (mV).

Scatter plot of total Luminescence versus hydrogen peroxide concentration.

A series of hydrogen peroxide solution having the concentrations of 0.05- 150-µMol $.L^{-1}$. Using the optimum parameters that have been already established in previous section. Figure10 -A show the total luminescence vs. time profile for some of the used concentration. In addition figure 10-B show the total luminescence emission intensity versus hydrogen peroxide concentration that have a correlation coefficient of 0.9985 for the range of concentration ranging from 0.1-100 µMol $.L^{-1}$. Details of the obtained statistical results are tabulated in table 1.

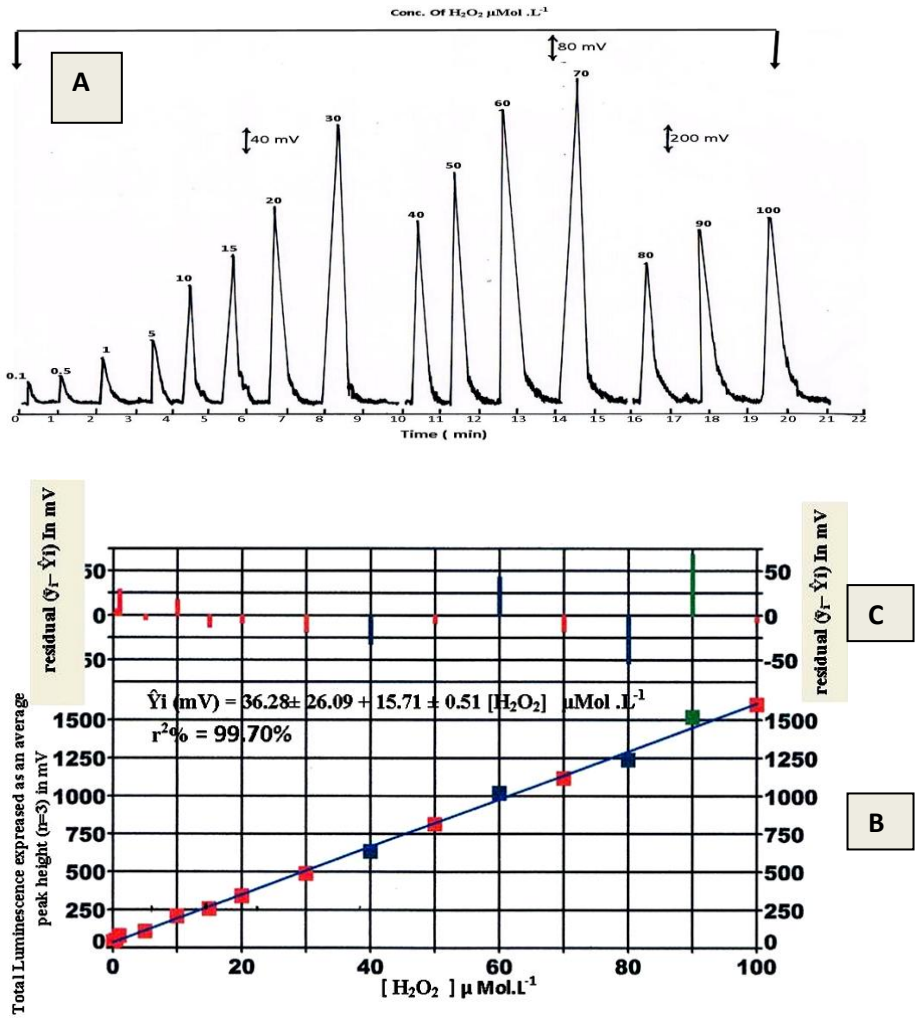


Figure 10-A: Variation of total Luminescence response versus H_2O_2 concentration
B: Effect of H_2O_2 concentration on the total luminescence expressed as an average peak height in (mV) by linear equation, C- residual ($\bar{y}_i$– Ŷi), $\bar{y}_i$: practical value, Ŷi: estimated value. Using 80µl, 5 µg.ml^{-1} Co(II) and [fluorescien] = 100 µMol .L^{-1} mixed with H_2O_2 ,as an injected sample .

Table 1-Summary of calibration graph results for the determination of H_2O_2 by total luminescence of H_2O_2- Lu $_{GB}$- Flu- Co (II) ion system.

Measured Conc. Of H_2O_2 (µMol .L^{-1})	**Linear dynamic range (µMol .L^{-1}) (n=15)**	**Linear equation Ŷi (mV) = a ± ts_a +b ± ts_b [H_2O_2] µMol .L^{-1} at confidence level 95% , n – 2**	**r , r^2 r^2%**	**t_{tab} at 95% confidence limit**	**t_{cal} $t=\frac{\lvert r\rvert\sqrt{n-2}}{\sqrt{1-r^2}}$**
0.1 –150	0.1 – 100	36.28± 26.09 + 15.71 ± 0.51 [H_2O_2] µMol .L^{-1}	0.9985, 0.9970 99.70%	2.16<< 66.08	

Ŷi (mV) =Estimated CL-F response for (n=3), [H_2O_2] in µMol .L^{-1}, r = correlation coefficient (C.C), r^2: coefficient of determination (C.O.D) r^2percentage = linearity percentage. t_{tab}= $t_{0.05/2, n-2}$ at 95% confidence level.

A value of linear correlation coefficient (r) which is equivalent to 0.9985 indicate strong positive correlation between the response measured by mV which is regarded as dependent variable and the concentration of hydrogen peroxide measured by µMol.L^{-1} which is regarded here as the independent variable . A value for coefficient of determination (C.O.D. = r^2) which is equivalent to 0.9970 indicate clearly that 99.70% i.e., the proportion of the variance (fluctuation)of the response that is predictable from concentration is very strong , it also represent the certainty of the predicted values from linear

regression equation i.e . The linear regression model (Response (mV)=36.28 + 15.71[H_2O_2] µM). This strong value of R^2represent the ratio of the explained variations (differences) to the total variations.

Limit of detection (L.O.D)

Using successive dilution of lowest used concentration in the calibration graph 217.60 pg / sample was the L.O.D of this used methodology

Repeatability

Repeatability of measurements was studied at two variable concentrations of H_2O_2 solutions at optimum parameters. The repeated measurements for six successive injections were measured and tabulated in table 2, while figure 11-A, B shows a kind of response-time profile for the used concentrations.

Table 2-Repeatability results of H_2O_2 at optimum parameters by CFIA-CL/fluorescence method

[H_2O_2] µMol .L^{-1}	**No. of injections**	**Total luminescence response expressed as average peak heights (mV)**	**Standard deviation σ_{n-1}**	**RSD %**	**Confidence interval of the mean at 95% $\bar{y}_i \pm t_{0.05/2,\, n-1} \times \sigma_{n-1}/\sqrt{n}$**
30	6	489	1.273	0.26	489 ± 1.336
50	6	813	1.874	0.32	813± 1.967

$t_{0.05/2,\, n-1}$= 2.571

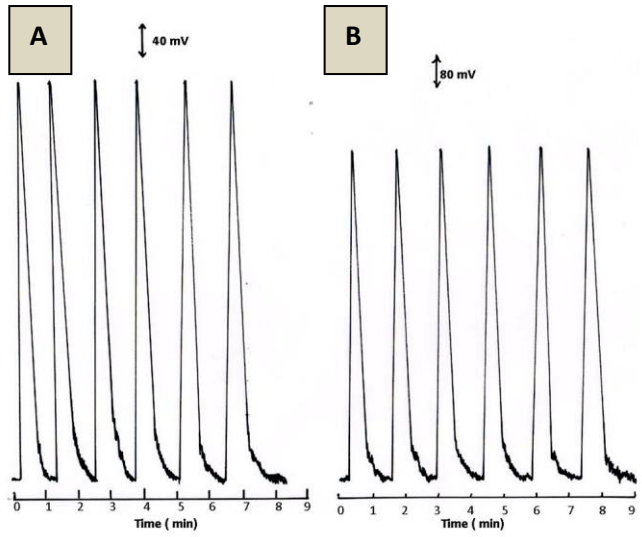


Figure 11-The total luminescence response -time profile for five successive repeatable measurements of H_2O_2; A- 30 µMol .L^{-1}, B- 50-µMol .L^{-1}

Analysis of pharmaceutical preparation:

The established method was used for the determination of H_2O_2 in three different kind of H_2O_2 samples from three different well known manufactures (Baghdad company- 25% ,Al-Amire company- 20% and Al-areje company- 7%) using total luminescence of H_2O_2- Lu $_{GB}$- Flu- Co (II) ion system. and compared with measured the attenuation of incident light (turbidity) as well as reflection of light at two opposite positions and algebraic sum of them [29] by a homemade Ayah $4S_W$-3D-T_{180} - $2N_{90}$ - Solar - CFI Analyser [30] for Determination of Hydrogen Peroxide by Cr (III) - OH^- - H_2O_2 - Ba (II) system . The standard additions method was applied by preparing a series of solutions from each pharmaceutical drug via transferring 0.1 mL (50 mMol. L^{-1}) to five volumetric flask (100 ml) , followed by the addition of (0 , 0.01 , 0.015 , 0.02 , and 0.025 mL) from 100 mMol.L^{-1} standard solution of H_2O_2 in order to have the concentration range from 0 – 25 µMol.L^{-1} for the preparation of standard additions calibration plot . The measurements were conducted by both methods.

Results were mathematically treated [31-33] for standard additions method and tabulated in Table. 3 at confidence interval of 95 %.

Paired t – test was used as shown in Table. 4 which shows a comparison – treatment of data for the obtained results from both methods with neglecting the difference of origin. It was noticed that there were a significant difference between two methods as shown in column 7 at 95% for the determination of H_2O_2 in pharmaceutical samples.

Table 3-Results of hydrogen peroxide determination in pharmaceutical samples by CFI -Total luminescence measurement method and CFI -Turbidity ($T_{(0-180)}$) measurement method .

No. of sample	Company, Country, Percentage%, $[H_2O_2]$ Mol $.L^{-1}$	percentage% $[H_2O_2]$ mol L^{-1} After standardization	Volume draw (mL) to prepare 50 mmol L^{-1} H_2O_2 in 250 mL	Volume draw (mL) to prepare 50 µmol L^{-1} H_2O_2 in 100 mL	Practical $[H_2O_2]$ µmol L^{-1} (n=3)	Practical $[H_2O_2]$ mol L^{-1} and percentage at origin sample	
						Total luminescence measurement	*Turbidity ($T_{(0-180)}$) measurement
1	Baghdad company (Iraq) (25%) 7.35 Mol L^{-1}	9.45% 2.78	4.49	0.1	49.82 ± 1.23	2.77 9.42%	2.75 9.35%
2	Al-Amire company (Syria) (20%) 5.88 Mol L^{-1}	1.74% 0.51	24.51	0.1	48.98± 1.34	0.49 1.69%	0.46 1.56 %
3	Al- Areje company (Iraq) (7%) 2.06 Mol L^{-1}	1.24% 0.37	33.78	0.1	48.82±1.07	0.36 1.23%	0.34 1.16 %

*Turbidity ($T_{(0-180)}$): measured via the attenuation of incident light by a homemade Ayah $4S_W$- 3D-T_{180} - $2N_{90}$ - Solar - CFI Analyser

Table 4-paired t –test results for total luminescence (proposed method) with classical method by Ayah $4S_W$-3D-T_{180} - $2N_{90}$ - Solar - CFI Analyser using standard additions method for determination of H_2O_2 in pharmaceutical samples.

Sample no.	Practical $[H_2O_2]$ mol L^{-1} and percentage at origin sample		Xd	□ d	σ_{n-1}	$t_{cal} = \frac{\bar{X}\ d\ \sqrt{n}}{\sigma_{n-1}}$ at 95%	*t_{tab} at 95%
	Total luminescence measurement	*Turbidity ($T_{(0-180)}$) measurement					
1	2.77 9.42%	2.75 9.35%	0.02	0.0233	0.0058	6.96 >> 4.303	
2	0.49 1.69%	0.46 1.56 %	0.03				
3	0.36 1.23%	0.34 1.16 %	0.02				

t_{cal} >> t_{tab} (4.303) at 95% , * $t_{tab} = t_{0.05/2}$, n-1 = 4.303

Conclusion:

The proposed method is simple, rapid and inexpensive with high sensitivity for the determination of H_2O_2 as an oxidizing agent of Luminol which is loaded on poly acrylic acid gel beads in the presence of Cobalt (II) ion as a chemiluminescence catalyst to release chemiluminescence light as an internal source for irradiation fluorescien molecule to give fluorescence light. This method can be used for the analysis of H_2O_2 in pharmaceutical disinfectants from the experiment point of view, the manipulation is very simple and sequential measurement was permitted with sample frequency up to 35 sample per hour. The proposed method used cheaper instruments and reagents. The R.S.Dpercentage less of than 1% was observed for all samples, indicating a satisfactory precision of the proposed method. The standard addition method was used to avoid matrix effects. Also this method can be applied to pico gram determination of Am in pure and pharmaceutical preparations .It offers the advantages of high sensitivity without the need for heating or extraction.

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