New Developed Analytical-Chemiluminometric (Sensitized) via junction cell and light energy transfer by 8 mm optical fibers for determination of Ibuprofen in Pharmaceutical drugs

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Abstract :

The study presents a sensitive, fast and easy assay method that was developed for determination of Ibuprofen via continuous mode flow injection analysis i.e. new noble chemiluminescence cell with specially designed unit cell in the prescence of optical fiber to measure a chemiluminescence light which generated depend on the oxidation of luminol (as internal source for irradiation) by hydrogen peroxide in the presence of Bi (III) ion as a catalyst metal ion and Ibuprofen as a sensitized to give chemiluminescence light , this reaction and analysis occur in a specially homemade designed cell. Experimental parameters that lead to optimum concentrations of all chemicals and physicals variables . A sample volume of 100 µL was used throughout the whole work. It was found that the linear working range was between 0.005-20 mmol. L^{-1}, with correlation coefficient of 0.9966 and limit of detection L.O.D. (S/N =3) 10.314 ng / sample by using step wise dilution of the minimum concentration that was achieved by the calibration graph. Repeatability of less than 0.5% for eight successive injections of 10 mmol .L^{-1} of Ibuprofen. The method was applied successively in determination of Ibuprofen in some pharmaceutical disinfectants. paired t-test shows that there was no significant differences between newly developed method and UV- spectrometry classical method at 95% confidence interval . Therefore, the analyst should be able to choose any method for determination of Ibuprofen in different drugs and different samples .

Key word: Chemiluminescence, Flow injection analysis, hydrogen peroxide, Ibuprofen

Introduction

Ibuprofen(fig.1), 2-[4-(2-methylpropyl)phenyl]propanoic acid (a white crystalline powder with a characteristic odor and slight taste), is probably one of the most successful drugs used worldwide for the treatment of mild to moderate pain and various inflammatory conditions[1]. The use of ibuprofen and its enantiomer in a racemic mix is common for the management of mild to moderate pain related to dysmenorrhea, headache, migraine, postoperative dental pain, spondylitis, osteoarthritis, rheumatoid arthritis, and soft tissue disorder. Due to its activity against prostaglandin and thromboxane synthesis, ibuprofen has been attributed to alteration of platelet function and prolongation of gestation and labor. Besides, ibuprofen can be fed to the natural environment through pharmaceutical industry wastes. The existence of ibuprofen in the nature can have traumatic effects on living organisms[2]. In fact, it can widely endanger human life and the health of the natural habitat. Therefore, researchers have intended to discover methods to remove ibuprofen from the environment or reduce its existence down to minimum [3]. Ibuprofen is not suitable for people who: are sensitive to aspirin or any other non-steroidal anti-inflammatory drug have, or have had, a peptic ulcer have severe heart failure. There are many techniques reported for the determination of ibuprofen in various samples include chromatography[4,5], HPLC[6-9], spectrophotometry[10], potentiometry[11],emission fluorescence spectroscopy[12] and electrochemistry[13,14].

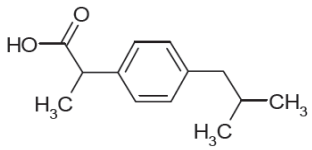


Fig .1: Chemical structure of Ibuprofen

Chemiluminescence , have been widely used for sensitive and selective detection in flow injection analysis which defined is defined as the production of electromagnetic radiation (ultraviolet, visible or infrared) observed when a chemical reaction yields an electronically excited intermediate or product, which either luminesces or donates its energy to another molecule, which then luminesces. This reaction has been attractive detection means for pharmaceutical analysis due to its low detection limits and wide linear working ranges, with relatively simple instrumentation[15-18] .

In this work describes a new Chemiluminescence cell [19] of a simple flow injection analysis for the determination of Ibuprofen , this reaction based on the oxidation of Luminol by hydrogen peroxide in presence of bismuth (III) ion as a chemiluminescence catalyst and Ibuprofen as a Sensitized . The designed cell can be measure a chemiluminescence(CL) light at Y- junction point when transfer to the PMT by optical fiber .

Experimental

Chemicals:

All chemicals were used of analytical-reagent grade while distilled water was used to prepare the solutions. A standard solution of 250 µg.ml^{-1} Bi (III) ion as Bi $(NO_3)_3.5H_2O$, BDH) was prepared by dissolving 0.5802 in 5 ml HNO_3 conc. And diluted to 1000 ml distilled water. A stock solution (1m 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 50 mmol.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. A stock solution of hydrogen peroxide H_2O_2 10mmol.L^{-1}) was prepared by pipetting 3.7 ml of hydrogen peroxide (20% vol., 34.01 g.mol^{-1}, BDH.) and complete the volume with distilled water to 500 ml volumetric flask. A standard solution 50mmol/L of Ibuprofen molecular formula $C_{13}H_{18}O_2$, molecular weight 206.285 g/mole and SDI-Iraq was prepared by dissolving 1.0314 g in 100 ml of distilled water.

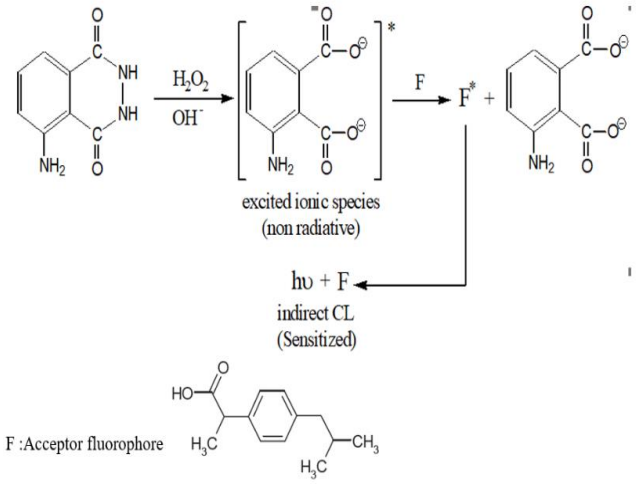
Apparatus

The flow system consist of : Peristaltic pump (Switzerland- Ismatic) . Rotary 6- port injection valve teflon,(USA)was used for loading and injection. The response was measured by a homemade a new Chemiluminescence cell (figure2) [19] for flow injection analysis. The cell design consist of two compartment mechanically separated completely ; except that light energy released in the first compartment is transferred via an optical fiber (8 mm) for a maximum distance 35 cm . This new design takes into account that reaction and receiving signal is done away from the processing of signal by a PMT supplied by high voltage in the second compartment . .The whole design was tested for luminol – H_2O_2 – Bi(III) – Ibuprofen system .The cell is completely protected from breakage and from light . PMT (RCA-2055 , VIS – NIR region) .

Methodology

Two lines manifold system was used as shown in fig 2 , the first line as a carrier stream of H_2O_2 (0.07 mmol/ L) at 1.5 mL min^{-1} flow rate which leads to the injection valve to carry the sample of Ibuprofen at 100 µl sample volume ; while the second line consists of a mixture from luminol (0.5 mmol/ L) with Bi(III) (5 µg.ml^{-1}) at flow rate of 1.5 ml.min^{-1} . These two lines were met and mixed at the junction point of new design of Chemiluminescence cell to release the Chemiluminescence light in front of beam of optical fiber. The obtained CL-response was recorded via photomultiplier tube (PMT) and the signals are recorded .

A proposed mechanism of chemiluminescence reaction explained in the Scheme 1 .



Scheme 1- proposed mechanism of Luminol / Bi(III) – H_2O_2 – Ibuprofen system

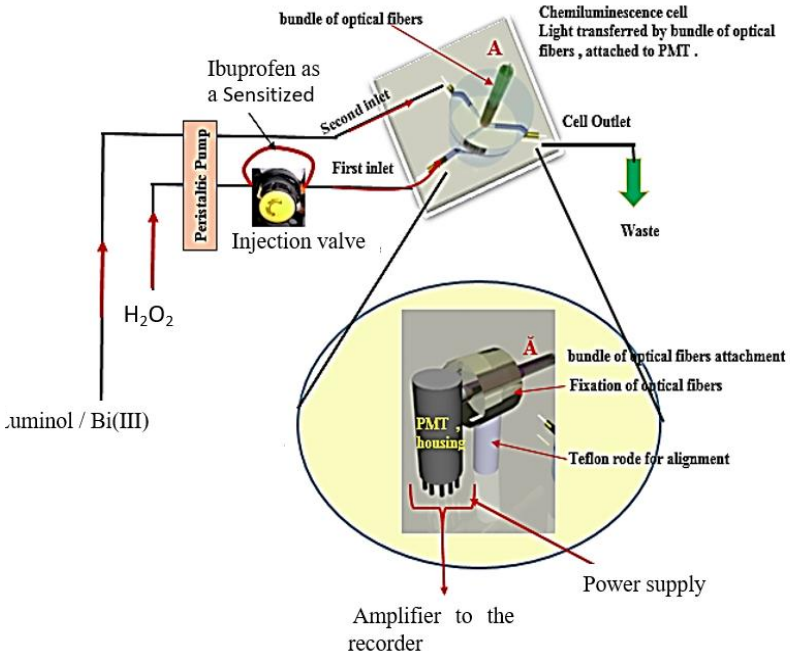


Fig . 2- schematic diagram for the determination of Ibuprofen by chemiluminescence reaction

Results and discussion

Optimization of variables

Chemical variables:

Preliminary sets of experiments were curried out to decide the optimum possible of chemical variable for determination of Ibuprofen . Variable concentration of luminol (0.1- 1) $mmol.L^{-1}$ in 0.1 mol/ L solution of Na_2CO_3 mixed with a constant concentration of Bi (III) ion at 0.5 $\mu g.ml^{-1}$ were prepared, and constant concentration of Hydrogen peroxide (0.01 $mmol.L^{-1}$). It indicate that at high concentration of luminol lead to deactivation of electronic excited states and saturation of electronic system . Therefore 0.5 $mmol.L^{-1}$ concentration of luminol a most suitable as a light source to provide the UV- light necessary for Ibuprofen to generated CL-emission . All of these effects are illustrated by the data in table no . 1 .and Fig 3.Variable concentration of hydrogen peroxide were used as an oxidant reagent for Luminol to generate the CL-emission at the presence of Bi (III) ion as a catalyst at ranging (0.01- 0.5) $mmol.L^{-1}$ using open valve mode with a sample volume of 75µL .. Table.2 tabulates the obtained results and Fig.4 show that 0.07 $mmol.L^{-1}$ of H_2O_2 was the optimum concentration to obtain high emission of chemiluminescence light as shown in Fig 4 and table no . 2 . While , the effect of Bi (III) ion concentration on Chemiluminescence light was study .Variable concentration of Bi (III) ion as a catalyst (0.5- 10 $\mu g.ml^{-1}$) mixed with 0.5$mmol.L^{-1}$ of luminol as a carrier stream in the first line of manifold design system and 0.07$mmol.L^{-1}$ concentration of Hydrogen peroxide in the two line was used as an oxidant reagent for Luminol .The sample volume of Ibuprofen as an acceptor fluorophore to generate the CL-emission is 75µl , using open valve mode . The results are shown in Fig 5 and table no. 3 , shows that the 5 $\mu g.ml^{-1}$ of Bi(III) ion is the most suitable concentration for maximum Chemiluminescence light.

Table 1 : Effect of variation of luminol concentration

[Luminol] mmol/L	CL – emission expressed as an average peak heights(n=3) $\bar{y}_i$ in mV	Confidence interval of the average response (95% confidence level) $\bar{y}_{i(mV)} \pm t_{0.05/2,\, n-1}\ \sigma_{n-1}/\sqrt{n}$
0. 1	342	342± 0.45
0.3	792	792±1.09
0. 5	994	994±1.04
0.7	903	903±1.11
1	800	800 ±0.78

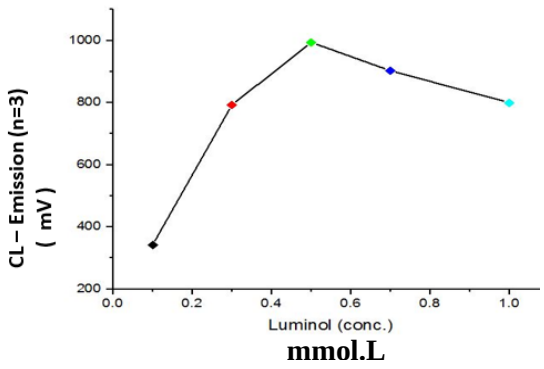


Fig 3. Effect of [Luminol] on CL- Emiss

Table 2 : variation of H_2O_2 concentration on CL- emission

$[H_2O_2]$ mmol/L	CL – emission expressed as an average peak heights(n=3) $\bar{y}_i$ in mV	Confidence interval of the average response (95% confidence level) $\bar{y}_{i(mV)} \pm t_{0.05/2,\, n-1}\ \sigma_{n-1}/\sqrt{n}$
0. 01	990	990±1.03
0.05	1100	1100±1.12
0. 07	1520	1520 ±1.07
0.1	1430	1430±1.09
0.3	1400	1400±1.12
0.5	980	980±1.14

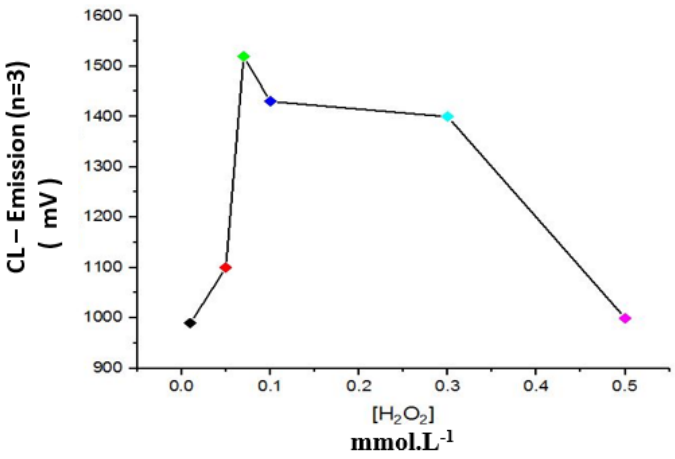
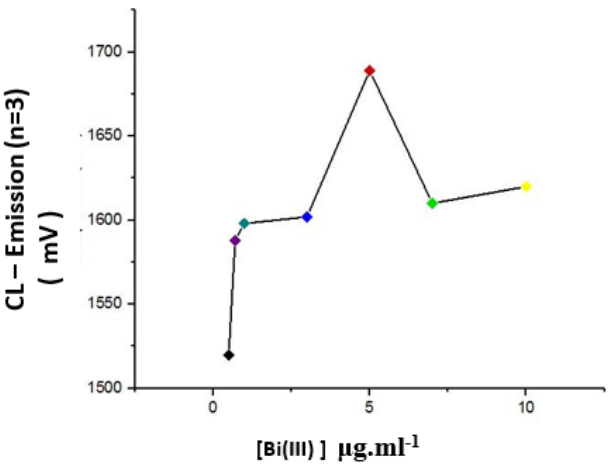


Fig 4. Effect of [H_2O_2] on CL- Emission

Table 3 : variation of Bi(III) concentration on CL- emission

[Bi(III)] µg.ml^{-1}	CL – emission expressed as an average peak heights(n=3) $\bar{y}_i$ in mV	Confidence interval of the average response (95% confidence level) $\bar{y}_{i(mV)} \pm t_{0.05/2,\, n-1}\ \sigma_{n-1}/\sqrt{n}$
0. 5	1520	1520±1.23
0.7	1588	1588±1.05
1	1598	1598±1.14
3	1602	1602±1.08
5	1689	1689±1.13
7	1610	1610±1.23
10	1620	1620±1.58



Physical Variables

Fig 5. Effect of [Bi (III)] on CL- Emission

Effect of Flow Rate & sample volume on CL- Emission

Using the optimum parameters achieved in previous section. Variable sample volumes (25- 200) µL were injected using open valve mode. The data obtained were plotted as shown in Fig. 6.A showing that the optimum sample volume is 100 µL given regular clear chemiluminescence response. Then followed by a decrease of CL – emission with the increase of sample volume i.e > 100 µL leading to width of their peak maxima which was most probably attributed to continuous long time duration of chemiluminescence light as shown in Fig.6 .A . The results are summed up in table 4. A . While at a variable flow rate as tabulated in table no.4.B. , I t was noticed that at low flow rate there is an increase in dilution and dispersion due to the diffusion of light segment in CL cell , leading to an increase of light segment which might cause an increase in base Δt_B as shown in Fig. 6,B. . while at higher speed > 1.5 ml.min^{-1} , the response was sharp in a maxima, but it is not very high due to the departure of the reactant from measuring cell prior to the completion of CL reaction therefore 1.5 ml.min^{-1} was used to obtain a maximum chemiluminescence light.

Table 4 : Effect of the variation of sample volume (A) & flow rate (B) on the CL – emission for the determination of ibuprofen using Luminol / Bi(III) – H_2O_2 – Ibuprofen system .

(A)

Sample volume (µL)	CL – emission expressed as an average peak heights(n=3) $\bar{y}_i$ in mV	Confidence interval of the average response (95% confidence level) $\bar{y}_{i(mV)} \pm t_{0.05/2,\, n-1}\ \sigma_{n-1}/\sqrt{n}$
25	1320	1320±1.13
50	1576	1576±1.07
75	1689	1689±1.42
100	1699	1699±1.16
150	1609	1609±2.03
200	1580	1580±2.09

(B)

Flow rate (mL.min^{-1}) For Two line	CL – emission expressed as an average peak heights(n=3) $\bar{y}_i$ in mV	Confidence interval of the average response (95% confidence level) $\bar{y}_{i(mV)} \pm t_{0.05/2,\, n-1}\ \sigma_{n-1}/\sqrt{n}$
0.9	1200	1200±1.05
1.2	1530	1530±1.13
1.5	1699	1699±1.07
1.8	1610	1610±1.95
2	1598	1598±2.09

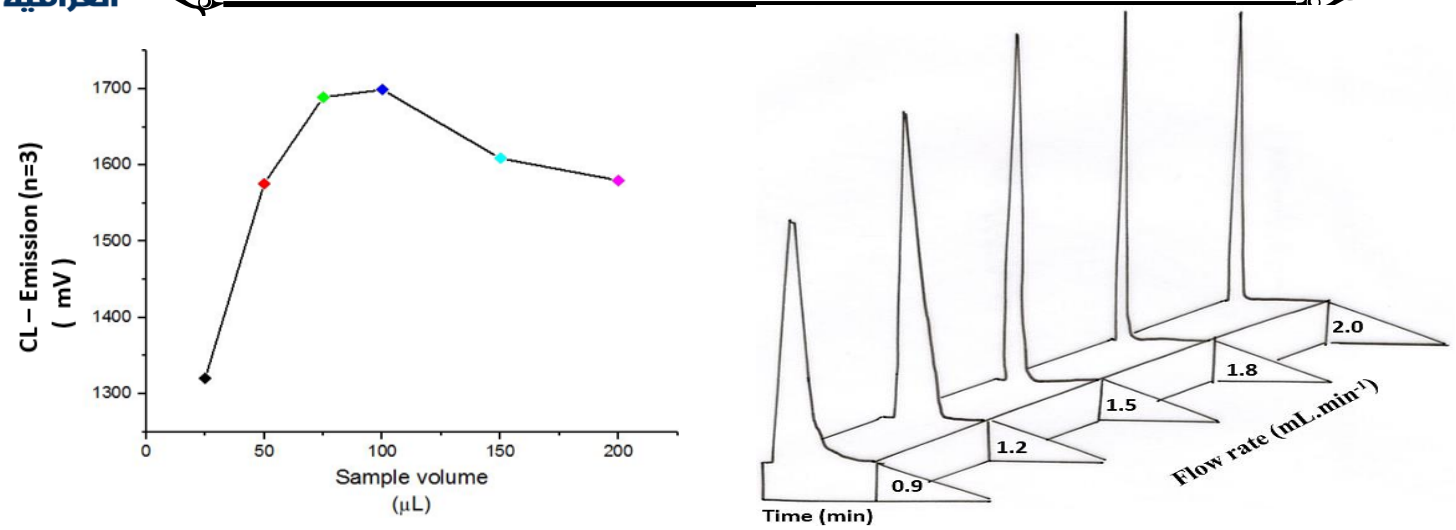


Fig . 6 : Effect of variation of the Sample segment on CL- emission expressed as an average peak heights (mV) (A) & Flow rate on CL- profile versus time **(B) for determination of ibuprofen using Luminol / Bi(III) – H_2O_2 – Ibuprofen system , 100 µL .**

Analytical range of chemiluminescence emission versus ibuprofen concentration.

A series of Ibuprofen solution having the concentrations of 0.005- 20 mmol.L^{-1}. Using the optimum parameters that have been already established in previous section. Figure No. 7 , show the chemiluminescence emission intensity versus concentration of Ibuprofen that have a correlation coefficient of 0.9966 for the range of concentration ranging from 0.005- 20 mmol.L^{-1} .In which a plot of the chemiluminescence emission intensity of solutions depends on concentration of the emitting species which in turn to depend on concentration of Ibuprofen So , at higher concentration (i. e , more than 20 mmol.L^{-1}) , the linearity of calibration graph is lost and chemiluminescence emission in mV then lies below an extrapolation of the straight – line plot . This might be attributed to self-quenching or self-absorption and the collisions between excited molecules lead to deactivation of energy .Linear regression equation with acceleration coefficient of determination (C.O.D) r^2 which is equivalent to 0.9931 was obtained as follow :

CL- emission (average peak height ($\hat{Y}i$)) in (mV)= 121.229 ±12.204 + 133.639 ±24.126 [Ibuprofen] mmol.L^{-1} for n= 14 at confidence level 95%

Using successive dilution of lowest used concentration in the calibration graph 10.314 ng / sample was the Limit of detection (L.O.D) of this used methodology

Repeatability of measurements was studied at 10 mmol.L^{-1} concentration of Ibuprofen solution at optimum parameters. The repeatability of less than 0.5% with σ_{n-1} of 2.150 mV obtained for eight successive measurements of 100 µL Ibuprofen , while figure No 8 shows a kind of response-time profile for the used concentration.

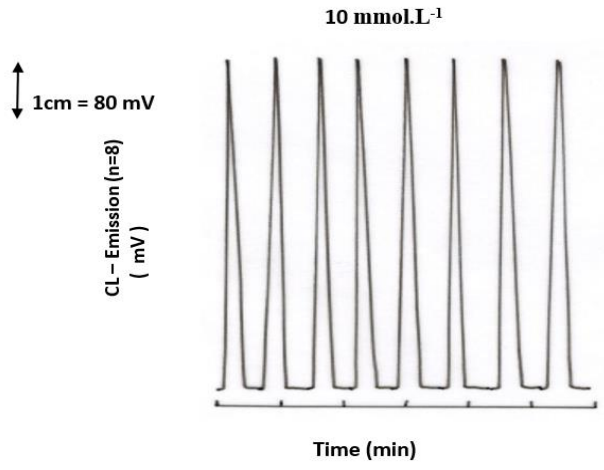


Figure No 8 : Chemiluminescence response -time profile for eight successive repeatable measurements of Luminol / Bi(III) – H_2O_2 – Ibuprofen system , 100 µL .

Analysis Ibuprofen in pharmaceutical drugs

The continuous flow injection analysis via chemiluminescence light response using a homemade a new Chemiluminescence cell and transfer beam of light by optical fibers achieved in this work was used for the analysis

of Ibuprofen in the four different drug manufactures (Profinal-UAE-400mg, jazofen-UAS-400mg, Apifen- India-200mg, and Ibuprofen- U.K- 200mg) and was compared with UV-spectrophotometric via the measurement of absorbance at λ_{max} = 220 nm by UV-1800, UV-spectrophotometer- Shimadzu, [20] .The method of measurement of chemiluminescence emission for **Luminol / Bi(III) – H_2O_2 – Ibuprofen system** was depends on the prepared of a series of solutions of each drugs (5mmol.L^{-1}) (0.10314 gm of active ingredient in 100ml) by transferring 0.02ml to each five volumetric flask (10ml), followed by the addition of gradual volumes of standard solution of Ibuprofen (0, 0.01, 0.02, 0.03, and 0.04 ml) of 5mmol/L to obtain (0.0 , 0.05, 0.1, 0.15, and 0.2 mmol/L). The results were summed in table 5 at confidence level 95% (2-tailed), showing practically content of Ibuprofen in each sample of drug using two different methods and efficiency of determination.

The developed newly adopted methodology in this research work was put into a paired t-test [21,22] for the sake of accepting it as an alternative method for analysis and assessment of Ibuprofen with standard used method. The obtained results indicate clearly that there was no significant difference between newly developed method (chemiluminescence - emission) with the classical UV-method [20] at 95% confidence level because calculated t value (3.203) ≅ tabulated t value (3.182) and there was no significant difference between two methods . Therefore, the analyst should be able to choose any method for analysis of Ibuprofen in different drugs .

Table 5. : Summary of results by standard additions method for the determination of Ibuprofen using chemiluminescence reaction

	me , content and company country	erval for the average $\frac{}{\sqrt{n}}$	equivalent to 0. gredient	ntent of the active $\frac{}{n}$	$\bar{y}_i$ (n=3) in mV eloped methodology) $\pm s_a t+b\pm s_b t$[folic acid] mmol /L					eter $\bar{y}_i$ (n=3) at (classical method) $b\pm s_b t$ [Ibuprofen] mmol /L		
					mmol/L 0.0	0. 05	0.1	0.15	0.2	standard addition at 95 $\pm s_b t$[x]		nt %
					$\bar{y}$ i for n= 3							
1	Profinal 400mg Julphar - UAE	0.6192 ±0.0025	0.15966	400 ±1.6149	21	110	208	310	388	20.60 ±4.33+ 1868± 89.15 [Profinal] mmol /L 0.9992 , 99.84%		0.011 , 5.50 440.013 , 110 %
										0.703±0.487+6.856±3.981 [Profinal] mmol/L		410.042 102.51 %
2	Jazofen 400mg Jena pharm -UAS	0.6245 ±0.0031	0.16103	400 ±1.9856	25	90	160	250	340	15.00 ±2.13+1580 ± 91.43 [[Jazofen] mmol /L 0.9970 , 99.41%		0.0094 , 4.70 379.75 , 94.94%
										.641±0.325+6.9220±2.857 [Jazofen] mmol/L		370.178 92.55 %
3	Apifen 200mg Zauba -India	0.2299± 0.00123	0.1186	200 ±1.0700	25	130	230	340	444	24.20±1.231+ 2096± 45.213 [Apifen] mmol /L 0.9999 , 99.98 %		0.012 , 5.77 230.837 , 115.42%
										0.727±0.395+ 7.016± 3.223 [Apifen] mmol /L		207.182 , 103.59%
4	Ibuprofen 200mg DHP Co. - U.K	0.2403± 0.0019	0.1239	200 ±1.5814	30	140	255	375	500	25.00±1.018+2350±45.32 [Ibuprofen] mmol/L 0.9997 , 99.94%		0.011 , 5.32 212.81 , 106.41%
										0.623±0.048+6.062±0.370 [Ibuprofen] mmol/L		205.608, 102.80 %

Conclusion.

A chemiluminescence – CFIA method is proposed and its applied successfully for analysis of Ibuprofen in different drugs or in blood and urine samples without any need for pretreatments.

The method based on oxidation of luminol (CL-donor) by hydrogen peroxide in the presence of Bi (III) ion as a catalyst metal ion and Ibuprofen as an molecule enhanced for the CL- reaction. The proposed method is simple, rapid and sensitive. An improved linearity and detection limit compared with the available literatures cited in the introduction .So ,the method described in this work offer a new alternative method for the determination of Ibuprofen .

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