FIA-CL DETERMINATION OF PARACETAMOL USING LUMINOL–$KMNO_4$–PB POST-CL SYSTEM, APPLYING MERGING ZONE PRINCIPLE

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(Accepted for publication: June 9, 2013)

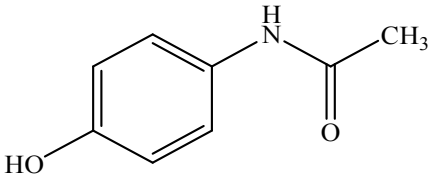
Abstract

This paper reports a new and simple flow injection analysis (FIA) with post- chemiluminescence system (PCL) for determination of paracetamol through merging zone principle. The method was based on the inhibition of luminol–$KMnO_4$–Pb post- chemiluminescence (PCL).Various parameters associated with this flow system were studied and essential optimizations were carried out. Calibration graph were constructed for determination of paracetamol in the range (5.0–30) µg.ml^{-1} with correlation coefficient (0.996) .The method was applied successfully for the determination of paracetamol in commercial pharmaceutical products.

***Keywords*: -** post-Chemiluminescence; flow injection analysis; paracetamol

Introduction

Paracetamol (PCT) is also known as acetaminophen (N-acetyl-p-aminophenol, 4-acetamidophenol) is a white or almost white crystalline powder, sparingly soluble in water, freely soluble in alcohol.



Paracetamol/ Acetaminophen

Paracetamol is part of the class of drugs known as aniline analgesics, it is the only such drug still in use today .Paracetamol was first marketed in the United States in 1953 by Sterling-Winthrop Co.,which promoted it as preferable antipyritic to aspirin since it was safe to take for children and people with ulcers. In 1963, paracetamol was added to the British Pharmacopoeia, and has gained popularity since then as an analgesic agent with few side-effects and little interaction with other pharmaceutical agents. Paracetamol is widely used for management of pain and fever.(Florey 2003)

Various analytical methods such as spectrophotometry,(Sultan *et al.* 2004; Idris *et al.* 2005; Afkhami *et al.* 2006; Shrestha and Pradhananga 2009; Nagendra 2011)fluorimetry,(Tavallali and Hamid 2011) flow injection analysis,(Oliveira *et al.* 2010) Flow-injection with chemiluminescence system.(Koukli and Hadjiioannou 1989; Alapont *et al.* 1999; Easwaramoorthy *et al.* 2001; Hua *et al.* 2002; Esmail 2004; Jabbar 2006; Ruengsitagoon *et al.* 2006; Zhao *et al.* 2006; Shu-min *et al.* 2011; Shi-qian 2011) electrochemical analysis, mass spectrometry, gas chromatography, capillary electrophoresis and liquid chromatography were employed for the determination of paracetamol. All these methods were used for determination of paracetamol either alone or in combination with other drugs.(Idris *et al.* 2005)

Some of these methods are less convenient for the determination of paracetamol in pharmaceutical formulations because the methods are based on the hydrolysis of paracetamol sample to 4-aminophenol, which then produced a coloured complex compound by an appropriate reaction which are time-consuming.

A new method was developed for the determination of paracetamol in which both flow injection and CL analysis were combined. The method was based on the inhibition of luminol-permanganate-pb post- chemiluminescence (PCL) with paracetamol by merging zone principle.

Experimental

Apparatus

The flow injection chemiluminescence system used in this work is shown in Figure (1). It consists of a peristaltic pump (Watson marlow205u) with 8 channels and variable speed regulator up to (10) ml/min to deliver flow streams. The silicon rubber pump tubes with (0.8) mm i.d were used to transport the solutions in the flow system.

Two six -way injection valves (knauer D-14163 berlin Nr.81521) and (cotati. California

Nr. 7125) with a sample loop of (40) µl were used to inject luminol and $KMnO_4$ into the flowing carrier streams. A Y-shaped Perspex piece was used to mix two streams of reagents.

The streams of luminol, $KMnO_4$, $pb(CH_3COO)_2$ and analyte were mixed in a flow cell positioned in front of the detector inside the spectrophotometer (spectronic CE303 GRATING spectrophotometer) the light source of which was blocked.

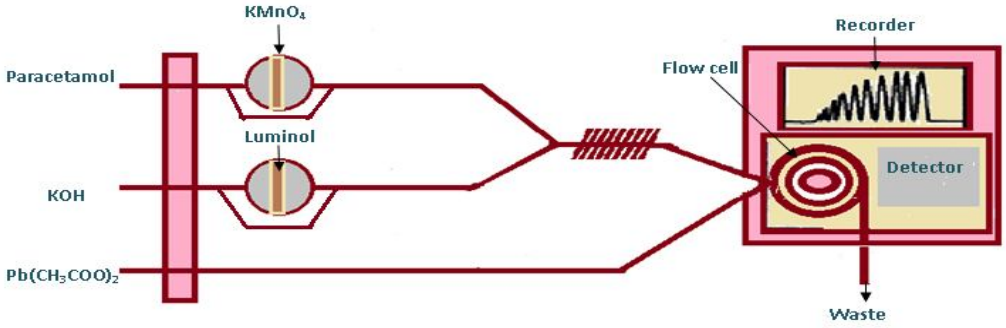


Figure (1): Schematic diagram of the FIA-CL manifold with merging zone principle used for the determination of pracetamol.

The chemiluminescent out-put was recorded by means of an x-t recorder (Type PM 825A PHILIPS — one line recorder) with various amplication factors and different chart speeds.

Reagents

Luminol solution ($1.0X 10^{-3}$) mole.L^{-1}

Luminol (5-amino-2,3-dihydro-1,4-phthalazinedione) solution was prepared by dissolving 0.1772 g of the solid (Surechem-LTD) in a little of 0.1 mole.L^{-1} sodium carbonate solution and the volume was completed to 1.0L in a volumetric flask with the same solution. The pH of this solution must be justified at (10 – 10.5).Other diluted solutions were prepared by serial dilutions using carbonate buffer solutions.

Potassium hydroxide solution (1.0) mole.L^{-1}

Potassium hydroxide solution was prepared by dissolving 56.11 g of potassium hydroxide (Riedel-Df Haen) in a little of water; the volume was completed to 1.0L in a volumetric flask.

Potassium permanganate solution (0.2) mole.L^{-1}

A (0.2) mole.L^{-1} $KMnO_4$ solution was prepared by dissolving (31.608) g of potassium permanganate (Hoplins and Williams) in a little of water. The solution was boiled for (15) min., then cooled and the volume was completed to (1.0) L in a volumetric flask. This solution was standardized against (0.1) mole.L^{-1} standard sodium oxalate solution.(Vogel 1979)

Lead acetate solution (0.1) mole.L^{-1}

A (37.9) g of lead acetate (Alpha) dissolved in (500) ml of distilled deionized water and make up the volume to (1.0) L.

Paracetamol stock solution (100) mg.L^{-1}

Stock solutions of (100) mg.L^{-1} paracetamol (S.D.I.) were prepared daily by dissolving (0.1) g of paracetamol in distilled deionzed water and diluting to (1.0) L, in a volumetric flask. Working standard solutions were prepared by serial dilution to obtain standard solutions for constructing calibration curves.

Solutions of interfering species (1) mg.L^{-1}:-

A stock solutions of each interfering species was prepared by dissolving (0.1) g of each interfering in (100) ml Distilled deionized water. Other solutions were prepared by the addition of different amounts of each interferent to a constant paracetamol concentration, and comparing the emission intensity with that form a sample with no interferent.

Sample preparation

Table (1) illustrates paracetamol contained in pharmaceutical formulations, which analyzed by the proposed FIA-CL method.

Tablet

Twenty tablets were weighed to obtain the average weight. They were grounded into fine powder and carefully mixed. A portion of powder, equivalent to one tablet (approximates mg of paracetamol), was accurately weighed, dissolved in distilled deionized water then transferred quantitatively to a (500) ml volumetric flask and the volume was completed with water to obtain a solution contain (1000)

$\mu g.ml^{-1}$ for tablets that contain (500) mg paracetamol and (900) $\mu g.ml^{-1}$ for tablets that contain (450) mg paracetamol. From this solution, other dilute solutions are prepared by appropriate dilution.

General procedure

As shown in Figure (1), solutions (40) µl of (5×10^{-4}) $mol.L^{-1}$ of $KMnO_4$ and (40) µl of (5×10^{-4}) $mol.L^{-1}$ of luminol were injected into carrier (15) $\mu g.ml^{-1}$ of paracetamol and (0.06) $mol.L^{-1}$ KOH streams which merge according to merging zone principle, controlling exact time so that the center of each injected slugs will meet the other, at the end of the luminal-$KMnO_4$ reaction later merges with another combined stream (8.0×10^{-3}) $mol.L^{-1}$ of Pb (II) in front of the detector to produce another post-CL signal. Paracetamol will react with $KMnO_4$ which leads to decrease the concentration of $KMnO_4$ in the flow stream and CL-intensity would decrease. The emission light was detected and the peak height of the signal recorded as a CL signal (mV).

Results and discussion

The FIA-CL configuration as shown in Figure (1) used for the determination of paracetamol. Reagent concentrations and manifold parameters were optimized for magnifying of paracetamol inhibition effect on the CL generated by luminol-$KMnO_4$-Pb(II) reaction.

Chemical, Optimizations

Effect of potassium hydroxide concentration

The effect of potassium hydroxide concentration on the chemiluminescence intensity was investigated over the range of (0.01 – 0.08) $mol.L^{-1}$. The optimum KOH concentration was (0.06) $mol.L^{-1}$ provided the maximum signal-to-blank ratio as shown in Figure (2). Thus, (0.06) $mol.L^{-1}$were selected for further experiments.

Effect of $KMnO_4$ concentration

The influence of $KMnO_4$ at different concentrations from (0.1×10^{-4} – 8.0×10^{-4}) $mol.L^{-1}$ were tested as shown in Figure (3). The peak height increased gradually with raising $KMnO_4$ concentration up to (0.5×10^{-4}) $mol.L^{-1}$, above which CL intensity decreased sharply probably because the $KMnO_4$ color at high concentration will obscure the CL transient emission.

Effect of Luminol concentration

The effect of luminol concentration on the CL intensity was investigated in the concentration range of (1.0×10^{-4} – 1.5×10^{-3}) $mol.L^{-1}$ and the results are shown in Figure (4). The optimum concentration of luminol for determination of paracetamol was (1.0×10^{-3}) $mol.L^{-1}$ that exhibits maximum signal-to-blank ratio. Therefore, this concentration was selected for subsequent studies.

Effect of lead acetate concentration

The concentration of pb(II) is an important factor , because it is used as an enhancer in the reaction. The influence of pb(II)concentration on the CL intensity was initially examined from (1.0×10^{-3} – 1.0×10^{-2}) $mol.L^{-1}$ as shown in Figure (5). The result indicated that (8.0×10^{-3}) $mol.L^{-1}$ pb(II) gave the highest relative CL intensity and hence selected for subsequent studies.

Physical, optimizations

Effect of the length of mixing coil

The effect of length of mixing tubing over the range (0-60) cm on the CL intensity was investigated as shown in Figure (6). It was found that (40) cm of the mixing tubing afforded the best results as regards sensitivity and reproducibility. Too short or too long mixing tubing can result in the decrease of CL intensity. Too short tube means the first CL reaction did not complete and too long leads to excessive dispersion. Therefore, (40) cm of the mixing tubing that gave achieve adequate mixing of the reactants was chosen for the subsequent studies.

Effect of the flow-rate

Effect of flow rates in the range of (0.5-7) ml/min were examined using the optimized reactant concentrations as shown in Figure (7) the signal increased with increasing flow rate up to (5) mL/min, above which the signal decreased. The present CL reaction is very fast and the excited product at the entrance of the CL reaction flow cell needs rapid transport to the reaction coil of the cell for maximum light output to be monitored, while at the flow rate higher than 5 ml/min the reactants leaving the flow cell and CL performed outside the detector optical path. Therefore, (4) ml/min was selected as the best flow rate.

Effect of reagents volume

Effects of different injected volumes of luminol and $KMnO_4$ (20 – 60) µl were studied using the optimized reactant concentrations and keep other physical variables constant. Figure (8) shows that the volume of (40) µl exhibits a good results as far as smooth peaks taken into

consideration and without fluctuation in the signals happened beyond this volume.

Calibration graph

Under optimum experimental conditions mentioned in Table (2), the calibration graph of the relative CL-intensity versus concentrations of paracetamol was obtained. The calibration graph was constructed by plotting CL intensity represented by peak height (mV) against paracetamol concentration ($\mu g.ml^{-1}$) as shown in Figure (9).

Statistical treatments of the calibration results including linear ranges, limits of detection, calibration equation and correlation coefficient for paracetamol are shown in Table (3).

To determine the accuracy and precision of the proposed method, four replicate determinations were made on the three different concentrations of standard paracetamol solutions. The accuracy was checked with a relative error (E %), while the precision of the method is checked with a relative standard deviation (RSD) of the same solutions. The results are shown in Table (4) which indicates good accuracy and precision.

Interferences

In order to assess analytical applicability of the method for paracetamol determination, the effect of some interfering substances which can be found in typical pharmaceutical preparations was tested by analyzing a standard solution of paracetamol (5) µg to which increasing amounts of interfering ions were added. The tolerable concentration ratios with respect to (5) µg of paracetamol for interference at (± 5) % level were listed in Table (5).

Application

The procedure was applied successfully for the determination of paracetamol in commercial pharmaceutical products. For the aim of comparison, the samples were also analyzed by HPLC as reference method(Godse *et al.* 2009).

The results are summarized in Table (6). A good agreement between the results obtained by the proposed method and reference method was observed.

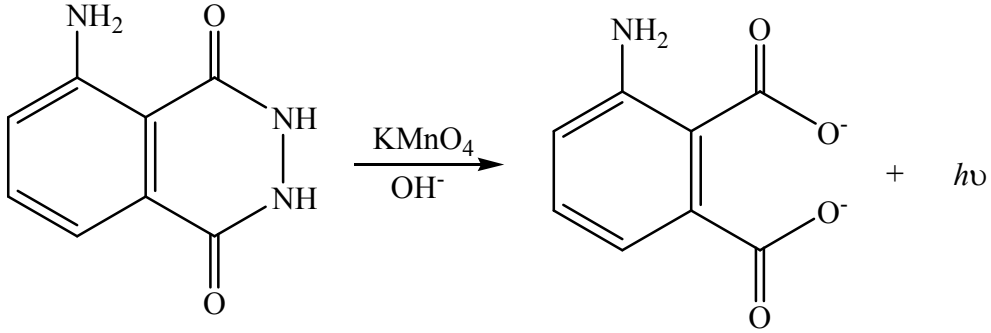
The results of proposed method and reference method are compared using the F-test and t-test. The student t-test and F-test show that there is no significant difference between the two methods with regard to accuracy and precision (t-calculated=0.72 < t-table =2.31 and F-calculated = 3.52 < F-table = 5.05 with a confidence limit of 95 %).

Accuracy

The accuracy of the proposed method was checked with a recovery R (%) of various amounts of paracetamol added to the respective pharmaceuticals. The results of the study are compiled in Table (8) which shows good accuracy.

Suggested mechanisim

The luminol–permanganate CL system emits weak CL in alkaline solution. as shown in this equation(Pan *et al.* 2007).

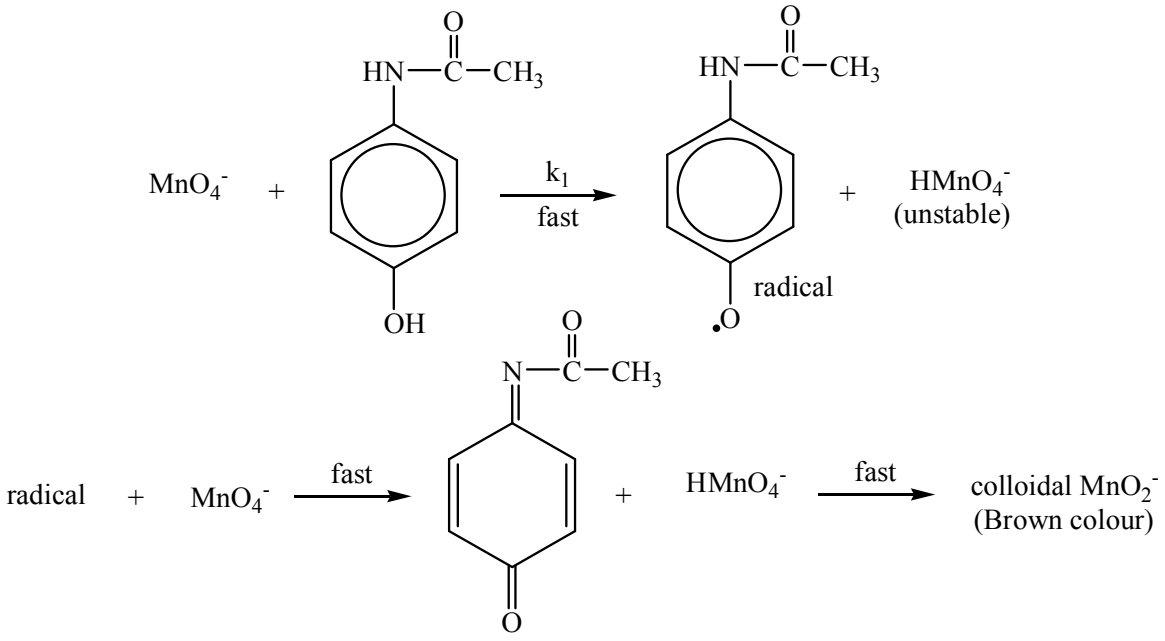


Nickel, mercury(II), lead, aluminum, alkaline earth metals and isoniazid can be detected by this post-CL phenomenon(Du J and Lu J. 2004 ,).

Potassium permanganate was firstly reduced by luminol to potassium manganate in alkaline medium, while luminol is oxidized to the excited species (3-aminophtalate ion). When the excited species returned to ground state and lost its energy by the emission of CL, the first CL signal occurred. By adding of lead ion the reaction between potassium manganate and 3-aminophtalate ion (that returned to ground state) activated to produce MnO_2 and excited species (3-aminophtalate ion) that returned to ground state and lost its energy by the emission of the second CL.

The CL intensity of the luminol–$KMnO_4$–Pb reaction is higher than that of the luminol–$KMnO_4$ reaction, so that the diverse effect of paracetamol will be more significant in this case.

Oxidation of paracetamol by permanganate in aqueous-neutral media is very fast at room temperature. Upon mixing aqueous solutions of permanganate and paracetamol, a readily distinguishable brown color appears due-to formation of water-soluble colloidal MnO_2.(Kumar and Khan 2006)



When paracetamol was present in the luminol–$KMnO_4$ system catalyzed by pb, the CL intensity decreased dramatically. The inhibition effect depends on the concentration of paracetamol.General equation of the total reactions can be illustrated as bellow:-

$$MnO_4^- + Pb^{+2} + \text{Luminol} \xrightarrow{\text{paracetamol}} \text{Quenching the Light}$$

Conclusion

The results presented in this work demonstrate that the coupling of luminol–$KMnO_4$-pb(II) post CL reaction monitored by an FIA method with merging zone principle is a very suitable approach to determine paracetamol residues at trace levels in pharmaceuticals, being a fast and cheap alternative.

The proposed method offers several advantages which are associated with the use of both FIA technique (low reagent consumption, high throughput and ease automation) and CL detection (high sensitivity, wide dynamic range and simple instrumentation).

In the present work, chemiluminescence generated by the reaction of luminol with $KMnO_4$ in basic media could be significantly enhanced by pb(II). This enhanced chemiluminescence was strongly inhibited in the presence of paracetamol. Based on these observations, a new flow-injection CL method was successfully proposed for the determination of paracetamol, in the range of 5-30 $\mu g.ml^{-1}$ with detection limits of (0.982) $\mu g.mL^{-1}$.

Luminol reacts with potassium permanganate to produce light emission in basic solution. Therefore, the alkalinity of the reaction medium is controlled by adjusting the KOH concentration in the luminol solution to improve the sensitivity of reaction.

The decrease in the signals at higher permanganate concentration is certainly due to the excess of permanganate, which overcasts the emitted light.

Luminol concentration plays a critical role in the CL reaction. Luminol was not only the reducing agent for the reduction of $KMnO_4$, but also the CL agent in the system. Both the CL signal and the blank signal increased as the luminol concentration increased. Hence, its concentration should be carefully optimized to ensure that the system was in good stability. In the present work, (1×10^{-3}) $mol.L^{-1}$ luminal gave the maximum blank to signal ratio above

this concentration the CL intensity decreased due to the self quenching of luminol molecules.

The concentration of pb(II) was an important factor, because it was used as an enhancer in the reaction.

Flow rate is a critical parameter in the flow-injection-based CL detection system. It influences the degree of diffusing and mixing of the reactants, determines the time that is taken from the final mixing point of the reagents (six-way valve) to the point that CL signal is detected (flow cell), and consequently influences the CL signal.

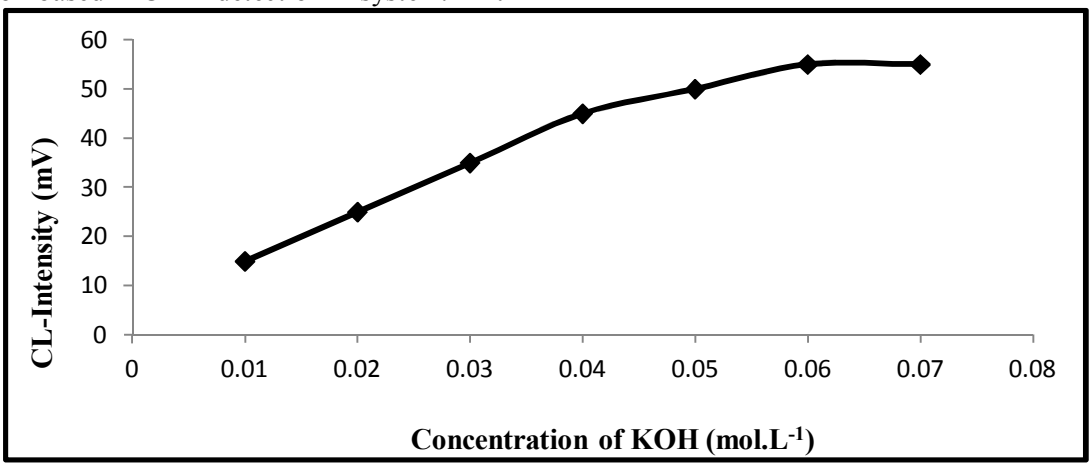


Figure (2): Effect of KOH concentration on the luminol–$KMnO_4$–Pb post CL-intensity in presence of 15 µg.ml^{-1} paracetamol

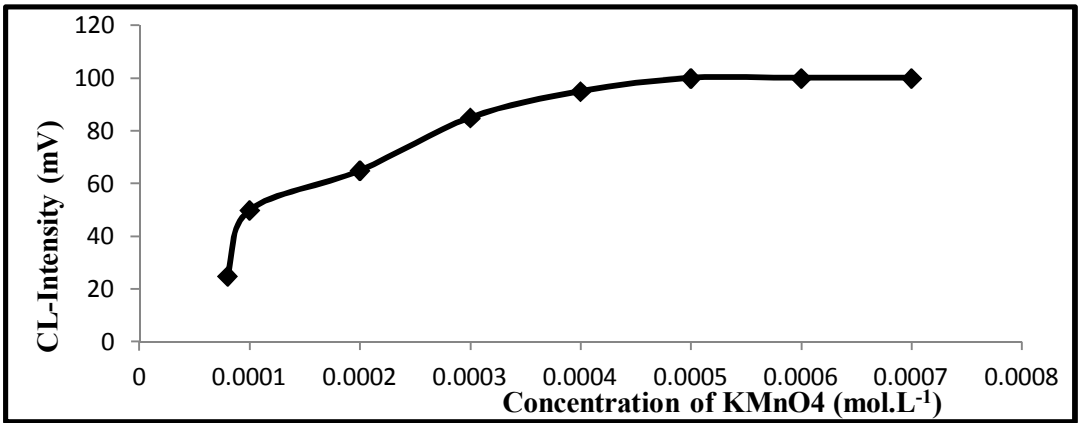


Figure (3): Effect of $KMnO_4$ concentration on the luminol–$KMnO_4$–Pb post CL-intensity in presence of 15 µg.ml^{-1} paracetamol

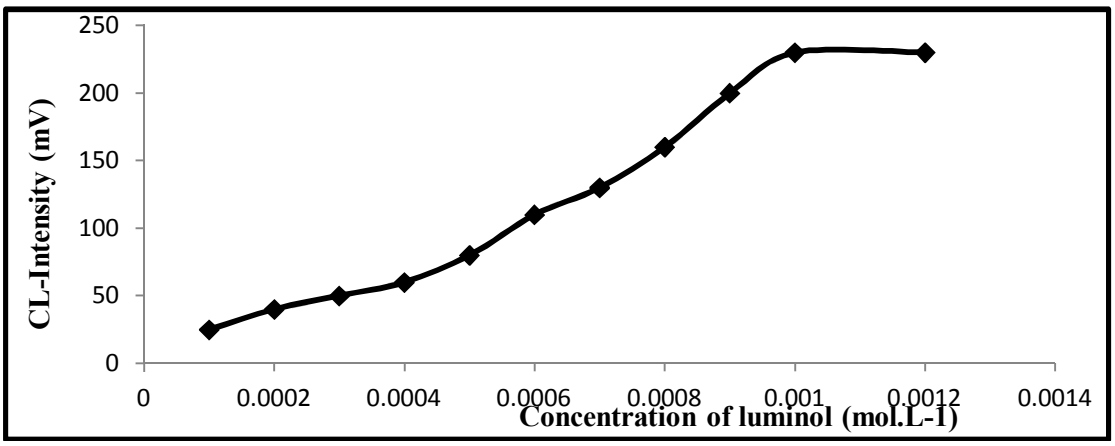


Figure (4): The effect of luminal concentration on the luminol–$KMnO_4$–Pb post CL-intensity in presence of 15 µg.ml^{-1} paracetamol

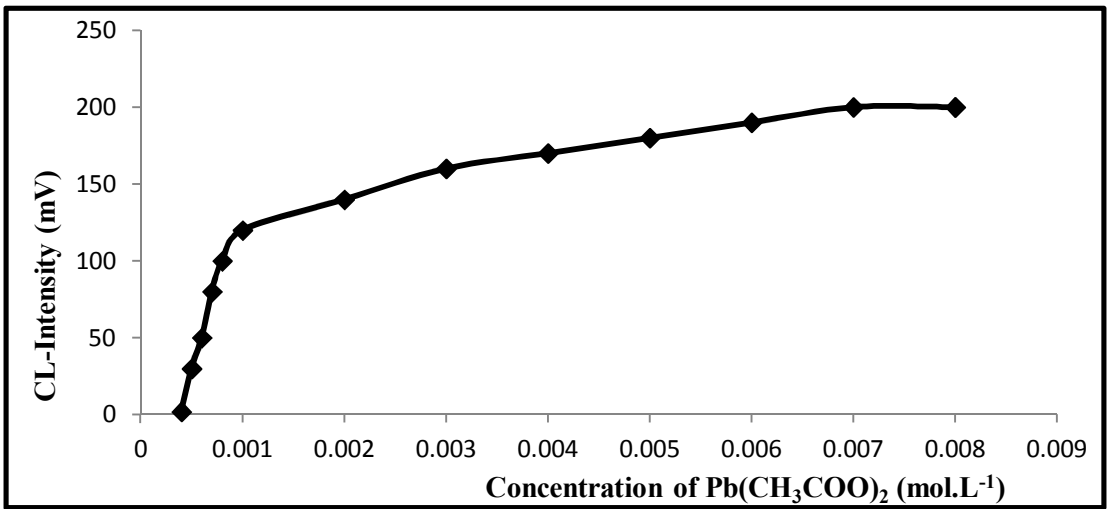


Figure (5): Effect of pb(II) concentration on the luminol–$KMnO_4$–Pb post CL-intensity in presence of 15 µg.ml^{-1} paracetamol

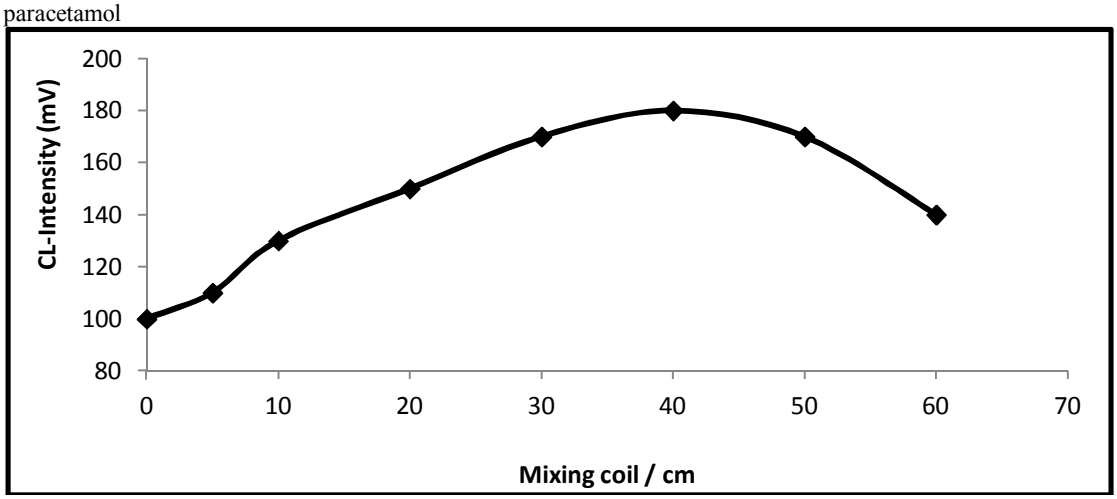


Figure (6): Effect of mixing coil on the luminol–$KMnO_4$–Pb post CL-intensity in presence of 15 µg.ml^{-1} paracetamol

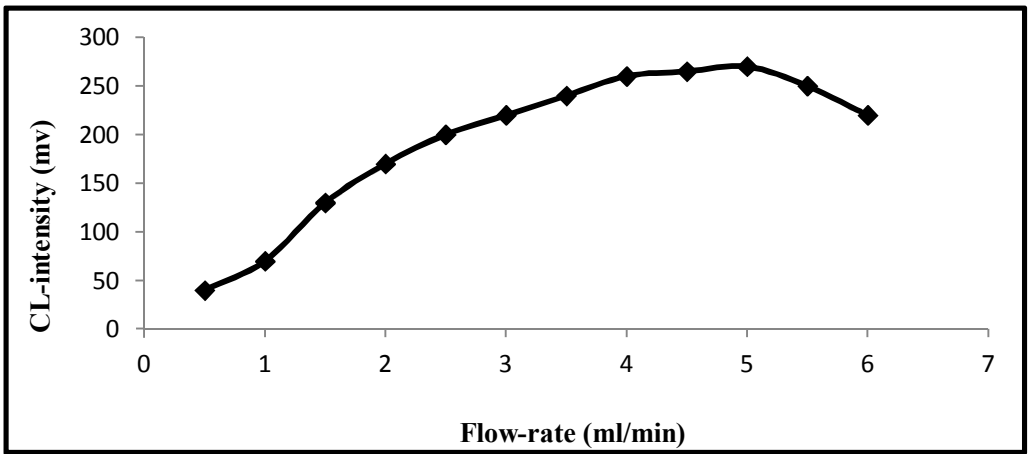


Figure (7): Effect of flow-rate on the luminol–$KMnO_4$–Pb post CL-intensity in presence of 15 µg.ml^{-1} paracetamol

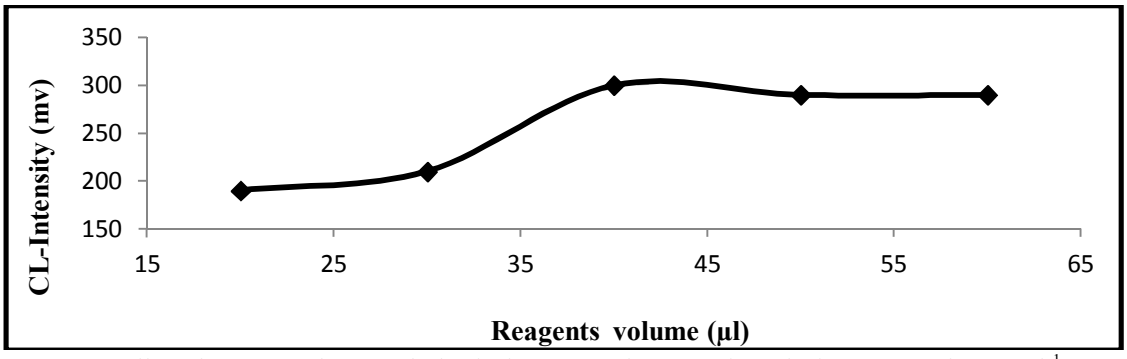


Figure (8): Effect of reagents volume on the luminol–$KMnO_4$–Pb post CL-intensity in presence of 15 µg.ml^{-1} paracetamol

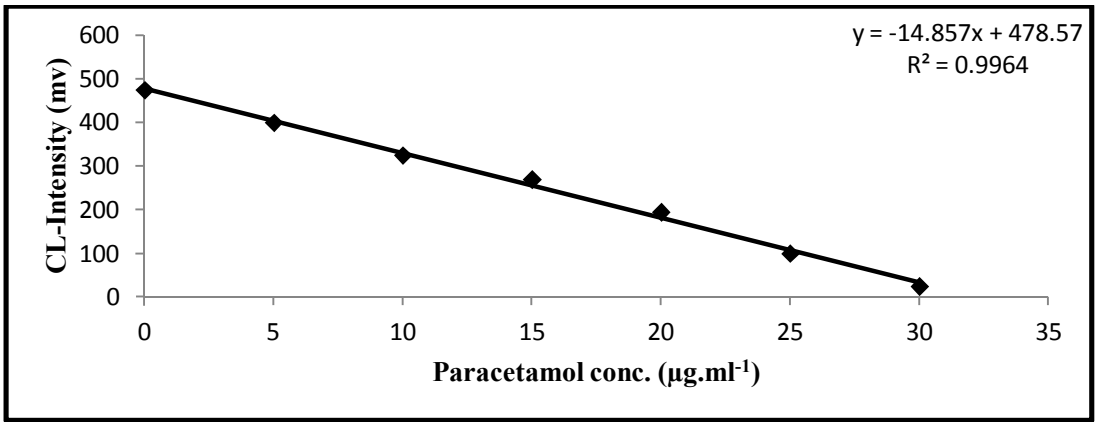


Figure (9): Calibration graph for the determination of paracetamol

Table (1): the trade name and composition of paracetamol contained in pharmaceutical

Trade name	company	composition	Labeled amount µg/tablet
Panda	Joswe medical	Paracetamol	500
		caffiene	65
myogesic	Dar al dawa, naur-jordan	Paracetamol	450
		Orphenadrine citrate	35
Kanawah-tablets	Kanawah-syria	Paracetamol	450
		Orphenadrine citrate	35
Reltef- tablets	China-mehcco pharmaceuticals and chemicals	Paracetamol	500
		Diclofenac sodium	50
		Chlorophenir amine malate	4
		Magnesium trisilicate	100
paracetamol	troge	Paracetamol	500

Table (2): Summary of optimum chemical and physical conditions for the determination of paracetamol.

Parameters	Optimum value
KOH concentration	0.06 mol.L^{-1}
$KMnO_4$ concentration	0.5×10^{-4} mol.L^{-1}
Luminal concentration	1.0×10^{-3} mol.L^{-1}
Pb(II)	8×10^{-3}
length of mixing coil	40 cm
Flow-rate	4 ml/min
reagents volume	40 µl

Table (3): Analytical data for determination of paracetamol

Compound	Linear rang ($\mu g.ml^{-1}$)	Correlation coefficient	Linear regression equation	Detection limit ($\mu g.ml^{-1}$)
Paracetamol	5-30	0.996	Y= –14.85x + 478.5	0.982

Table (4): Accuracy and precision of the present method

Compound	Conc. Of paracetamol ($\mu g.ml^{-1}$)	Mean (mV)	E (%)	SD	RSD%
paracetamol	5	395	1.25	9.1	2.3
	10	317	– 2.4	6.48	2.0
	20	191.25	– 1.92	4.77	2.4

Table (5) shows maximum tolerable concentrations of the various compounds.

Interference	Maximum concentration of interference ($\mu g.ml^{-1}$)	Paracetamol ($\mu g.ml^{-1}$)		Recovery%
		added	**found**	
Caffine	20	5	4.75	95
Orphenadrine citrate	10	5	4.68	93.75
diclofenac	10	5	4.81	96.25
Chlorophenir amine malate	10	5	4.75	95
Magnesium trisilicate	10	5	4.93	98.75

Table (6): Results of analysis of commercial drug formulations containing paracetamol by the proposed method.

Trade name	**mg/tablet**			**%E**
	Labeled amount	**Proposed method**	**Reference method**	
Panda Joswe medical	500	494.9	495	-0.02
myogesic Dar al dawa, naur-jordan	450	453	442	2.4
Kanawah-tablets Kanawah-syria	450	437	445	-1.79
Reltef- tablets China-mehcco pharmaceuticals and chemicals	500	486	493	-1.4
Paracetamol Troge	500	511	499	2.4

Table (7): Statistical analysis of (5.0) $\mu g.ml^{-1}$ of paracetamol using proposed method and reference method.

Trade name	Proposed method ($\mu g.ml^{-1}$) (d_2)	Reference method ($\mu g.ml^{-1}$) (d_1)	$dj = d_1 - d_2$	$(d_j - d^-)^2$
Panda Joswe medical	4.94	4.95	–0.01	17.64×10^{-4}
myogesic Dar al dawa, naur-jordan	5.03	4.91	0.12	77.44×10^{-4}
Kanawah-tablets Kanawah-syria	4.86	4.94	–0.08	12.54×10^{-3}
Reltef- tablets China-mehcco pharmaceuticals and chemicals	4.86	4.93	–0.07	10.40×10^{-3}
Paracetamol Troge	5.11	4.99	0.12	77.44×10^{-4}
			∑0.016	∑0.0401
		S_d=0.1		

$d^- = (1/n) \sum dj = 0.032$

d_1 and d_2 = value obtained by analyzing the analyte by the reference and Proposed method respectively.

d_i = sample mean of differences

Table (8): Recovery experiments for paracetamol added to sample solutions of commercial formulations.

Trade name	Initially present ($\mu g.ml^{-1}$)	Added ($\mu g.ml^{-1}$)	Found ($\mu g.ml^{-1}$)	Recovery (%)
Panda Joswe medical	10	3	13.25	101.9
		5	15.38	102.5
		10	20.21	101.05
myogesic Dar al dawa, naur-jordan	10	3	12.58	96.7
		5	14.71	98.06
		10	19.98	99.9
Kanawah-tablets Kanawah-syria	10	3	12.49	96.07
		5	14.26	95.06
		10	19.56	98.02
Reltef- tablets China-mehcco pharmaceuticals and chemicals	10	3	13.70	105.3
		5	14.93	99.5
		10	20.32	101.6
Paracetamol troge	10	3	12.47	95.92
		5	14.93	99.53
		10	20.55	102.75

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خەملاندنا باراسیتامول بشیکاریيا بریسکەی کیمیای بریبازا دەرزی لێدانا رووبشتوُ

پوختە

بریتیە ژرێکەکا نوی بۆ خەملاندنا پاراسیتامول لسەر بنەمایێ بەربەستکرنا لومینول – پرمانگەنێت – Pb بەریقەدارا پێشکەوتوو دگەل پاراسیتامول لسەر بناغێ دەڤەرا تێکەلچویی. دڤی کاری دا CL دەرکەفت بریکا کارلێکەرا لومینول دگەل KMnO4 دناڤەندا تفت دا ئەوا برەنگەکێ دیار گەشەدکەت بتێکرنا P(II).

ئەف گەشەکرنا CL گەلەك بهێز دئێتە بەربەستکرن بهەبونا پاراسیتامول. لسەر ڤان بنەمایا رێکەکا نوی یا کوتانا رویشتووی -CL بسەرکەفتیانە هاتە راسپاردن بۆ خەملاندنا پاراسیتامول، بخێچەکا راست دمەودای (5 – 30 مایکروگرام/مل) وهوکارێ بەراوردکرنێ (0.996) و سنورێ دیتنێ (0.982 مایکروگرام/مل).

تقدير الباراسيتامول بتقنية الحقن الجرياني العكسي مع البريق الكيميائي باستعمال نظام لومينول– برمنغنات – Pb

الملخص

استخدمت طريقة تقنية بسيطة للتدفق الجرياني العكسي مع البريق الكيميائي لتقدير الباراسيتامول . تعتمد الطريقة على قياس انخفاض شدة البريق الكيميائي الناتج من تفاعل اللومينول–برمنجنات البوتاسيوم – خلات الرصاص بواسطة الباراسيتامول حيث ان مقدار الأنخفاض في شدة البريق الكيميائي يتناسب طرديا مع زيادة تركيز الباراسيتامول.تم ضبط الظروف المثلى للتفاعل ومناقشة الية التفاعل.المنحني القياسي تتراوح بين 5 – 30 ميكروغرام/مل مع معامل الارتباط 0.996 . كما كان حد الكشف 0.982 ميكروغرام/مل من الباراسيتامول .