FLOW INJECTION DETERMINATION OF PARACETAMOL IN PHARMACEUTICAL FORMULATIONS USING LUMINOL – DIPERIODATOARGENTATE (III) SYSTEM WITH CHEMILUMINESCENCE DETECTION

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Abstract

A simple flow injection-chemiluminescence (FI-CL) method has been developed for the rapid and sensitive detection of paracetamol (PCM) in pharmaceutical formulations. Utilizing luminol-diperiodatoargentate (III) (DPA) system and optimizing all parameters, we attained an effective linearity range of 0.075-0.75 mg L^{-1}, with a coefficient of determination (R2) of 0.9999. The method showed a limit of detection (LOD) of 7×10^{-4} mg L^{-1}, a limit of quantification (LOQ) of 2×10^{-3} mg L^{-1}, and relative standard deviations (RSDs) ranging from 0.3-1.1%, which indicated high precision. Furthermore, the method achieved a high injection throughput of 120 h^{-1}, which showed it was suitable for real-world applications. Validation through comparison with a reported FI-CL method using the paired Student's t-test shown no significant differences at the 95% confidence level, and the method was successfully applied to determine PCM in pharmaceutical tablets.

Keywords: Flow injection; chemiluminescence; luminol; diperiodatoargentate (DPA); tablets

1. Introduction

Paracetamol (PCM) (acetyl-p-aminophenol) used on large scale as a pain reliever [1]. It was synthesized for the first time by Josephe Morse in 1878 and was introduced by Von Meering as an antipyretic and analgesic drug in 1893 [2, 3]. Initially, Phenacetin was preferred to use over PCM. As it was discovered that PCM was the primary metabolite of phenacetin with better tolerance, it replaced the usages of phenacitin in 1950s [4 - 6] has been widely used around the world as an analgesic and antipyretic drug over the counter (pain reliever & fever reducer) since then [7]. Prostaglandins act as mediator in inflammatory pain and fever [8, 9]. Its production is inhibited in the central nervous system using PCM. It is considered first line treatment for mild to moderate pain and fever [10, 11]. However, overdosing can cause liver toxicity or hepatic failure [12, 13]. To avoid the side effects of overdoses, it is imperative to determine the level of PCM in pharmaceutical and biological samples [14].
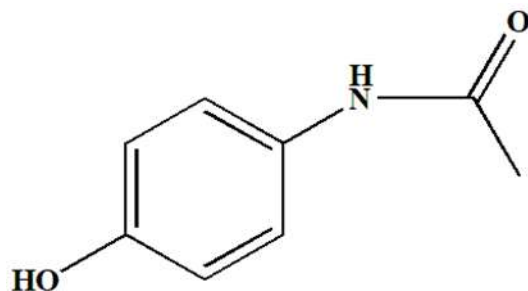
Figure 1. The structure of Acetaminophen

Various approaches have been employed to develop methods for PCM detection in pharmaceutical and biological samples, such as reversed phase high performance liquid chromatography (RP–HPLC)[15, 16] , ultra-performance liquid chromatography (UPLC)[17], gas chromatography–mass spectrometry (GC–MS) [18], spectrometry[19, 20], voltammetry[21, 22], capillary electrophoresis [23, 24], flow injection-chemiluminescence (FI–CL)[25, 26].Some of the above-mentioned methods are sensitive and precised, however, their procedures take a long time, need greater volumes of solvents, sample preparation has various steps, and analyses take longer time. FI–CL methods are also available but limited. Therefore, the detection of paracetamol in pharmaceutical and biological samples needs a quite simple, sensitive, fast and specific analytical method.

Chemiluminescence is a method in which enough energy (in the form of electromagnetic radiation mainly over the visible and near infra-red regions) and is produced during chemical reaction due to the electron transition from higher state to the ground state of excited intermediate product[27].This technique is commonly employed for detecting the quantity of a substance owing to its favorable features such as simple and cost-effective instrumentations, high sensitivity with low detection limit, rapid sample processing times, smooth automation compatibility, inexpensive dynamic response spectrum and minimal sample volume requirements [28 - 30].The combination of FI and CL detection methods produce cost-efficient, robust methods suitable for the analysis of various substances. Numerous studies have confirmed the broad applications of these combined methodologies across chemical analyses [31 - 33]

Luminol is amongst the most often employed Cl reagents [34]. It is oxidized by various oxidizing agents such as permanganate (MnO_4^{2-}), hypochlorite (ClO^-), periodate (IO_4^{2-}), and hydrogen peroxide (H_2O_2). Due to oxidation, luminol produces an excited state product called 3-aminophthalate, which emits blue light with the largest lambda of 425 nm. Moreover, other high and uncommon oxidation state transition metal complexes including diperiodatocuprate (III) (DPC), diperidoatonicklate (IV) (DPN), and diperidodatoargentate (III) (DPA) are increasingly being applied for analytical applications [35]. These compounds have been used under different conditions either in basic or acidic conditions. DPA is stable compound due to having square- planer coordination around silver in basic medium. DPA has mostly been used in basic medium, it has extremely limited studies to be used in direct oxidation in chemiluminescence analysis [36]. Yang et al. has used DPA in direct oxidation for the development of Cl system to determine uric acid in basic medium. Particularly, the DPA-luminol CL reaction has proven to be remarkably successful for the detection of various substances in different sample matrices when applied in flow systems [33, 37]. Several methods have been proved for the determination of PCM in various kinds of samples using FI with CL and other methods [15 - 26].

This study aimed to propose an approach for the detection of PCM in pharmaceutical tablets depending upon the enhancement of DPA and luminol reaction having good linearity and detection limit of 7×10^{-4} mg L^{-1}. The suggested method's reaction mechanism has been explored and discussed thoroughly.

2. Experimental

2.1 Chemicals

Deionized water was used to prepare all the solutions and all the reagents and chemicals employed were of analytical grade and employed without purification. PCM bulk was obtained from DANAS Pharmaceutical Ltd Islamabad. The PCM stock solution (1000 mg L^{-1}) was prepared by weighing 0.01g to dissolve into 10 mL of absolute ethanol. The PCM stock solution was used to prepare working standard solutions through dilution method in 0.1 % of ethanol.

Potassium hydroxide (KOH) was obtained from Merck Germany and KOH (0.1 M) stock solution was prepared to weigh 1.4 g of KOH and dissolved into 250 mL deionized water and used throughout experiment. Luminol was obtained from Sigma Aldrich USA and the luminol (0.01 M) stock solution was prepared in 0.01 M of KOH. The luminol stock solution was diluted to prepare working standard solutions in 1×10^{-3} M KOH solution. On the other hand, 1.0×10^{-3} M of KOH was prepared by taking 1 mL of 0.1 M KOH into 100 mL deionized water.

Diperiodatoargentate (III) (DPA) was synthesized by following the protocol reported previously [38]. The DPA synthesis was confirmed by the spectra showing two bands (362 and 252 nm) of UV-visible spectrometer. The DPA solution concentration was detected through spectrophotometer at 362 nm (ε = 1.26 10^4 M^{-1} cm^{-1}). The 4.7×10^{-6} M stock solution of DPA was prepared by dissolving 0.01 g of DPA in 8×10^{-3} M KOH solution. The 8×10^{-3} M of KOH was prepared from 0.1 M stock solution of KOH by taking 8 mL in 100 mL deionized water. Working standard solutions were prepared on daily basis of DPA compound in basic medium as per requirement (1.0×10^{-3} M of KOH solution). The stock solutions of various organic compounds were prepared by dissolving 0.01 g of each compound into 10 mL absolute ethanol. These organic compounds include sucrose, caffeine, glucose, fructose, lactose, starch, citric acid and ascorbic acid. All these compounds were obtained from Merck. The working standard solutions of the above-mentioned organic compounds with concentrations range of 0.1 mg L^{-1}, 0.5 mg L^{-1}, and 1.0 mg L^{-1} was prepared from the stock solution of each organic compound by the dilution with 0.1% v/v concentration of ethanol.

2.2 Sample Preparation

The commercial tablets of PCM of three varied brands (Panadol, 500 mg; Calpol, 500mg; Actified P-cold, 500 mg) were sourced from a local pharmacy in Quetta and brought to the laboratory. Subsequently, each tablet was weighed and then 10 tablets from each brand were finely powdered utilizing pestle and mortar. The average weight equal to one tablet of 10 crushed tablets was taken and were dissolved in 10 mL of anhydrous ethanol and sonicated for 15 minutes with 3000 rpm according to earlier method with slight changes [39]. The solution was then cooled and filtered; the filtrate was then diluted up to the mark in a 100 mL flask using 0.1 % v/v ethanol. Later, working solutions were prepared in 0.1% v/v ethanol, which also served as the carrier. The samples were later analyzed using a proposed and a previously reported method [26].

2.3 FI–Cl apparatus and procedure

Figure 2 depicts the FI–Cl apparatus employed for this experiment. The manifold holds several components, including a Swiss-made peristaltic pump, an Anachem-supplied six-port Rhodyne 5020 valve from Anachem in the UK, THORN EMI's model 9798B photomultiplier tube, polytetrafluoroethylene tubing from Fisher Scientific, an MP20SN power supply from THORN EMI in the UK and Kipp & Zonen's BD40 strip chart recorder from the Netherlands. All these components of the manifold were connected by making sample using polytetrafluoroethylene tubings. All the reagents were propelled to the PMT with 5.0 ml min^{-1} flow rate through peristaltic pump. PCM standard / samples having 0.1% v/v ethanol as a carrier (C) were injected through six-port Rhodyne 5020 valve. The stream of DPA (4.7×10^{-6} M in 8×10^{-3} M KOH) (R-1) was combined luminol (1.0×10^{-5} M in 1.0×10^{-3} M KOH) (R-II) at T-junction with stream before the entrance of PMT connected with power supply of 850 volt. The obtained Cl intensity was recorded using chart recorder, and the peak height was related to voltage output obtained from PMT (mV).
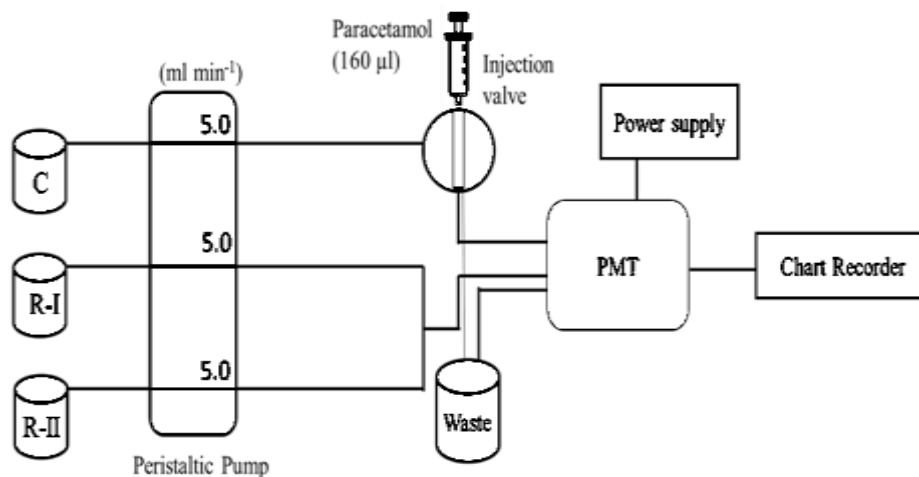
Figure 2. The schematic diagram of FI-CL Manifold for determination of PCM. C, Carrier 0.1% v/v ethanol; R-1, 4.7×10^{-6} M DPA in 8×10^{-3} M KOH; R-II, 1.0×10^{-5} M luminol in KOH 1.0×10^{-3} M; sample loop volume, 160 µl, PMT (Photomultiplier tube), 850 V.

2.4 Validation of suggested analytical approach

The suggested method employed for the detection of PCM level in pharmaceutical tablets was confirmed using an established two channel FI–Cl system (26). In this dual system, R_1 and R_2 was combined and had Ru (bpy) $_3^{2+}$ (6.4×10^{-4} M) and manganese (II) (1.25×10^{-2} M) in the ratio of 100:20. The optimized flow rate of this channel was set 1.5 mL min^{-1} and 100 µl of sample volume was used. Later, the stream was merged with R_3 stream having KMnO$_4$ ($7.0\ 10^{-4}$ M in H$_2$SO$_4$ 0.1 M) before the reaction coil (160 cm) at 0.5 mL min^{-1} flow rate. Standard solutions of PCM ranging from 0.1 mg L^{-1} to 0.5 mg L^{-1} was prepared from 1000 mg L^{-1} stock and then injected into the FI system. Calibration curves were plotted according to the signal's responses. Pharmaceutical tablets having PCM were then examined, their content determined using regression equations and dilution factors before being compared with results obtained through flow method analysis.

Precision and the accuracy of the suggested FI–CL system was evaluated using spiked samples, while statistical analysis was also performed including both paired student *t*-tests and *F*-tests to compare outcomes from two distinct methods.

3. Results and Discussion

3.1 Kinetic Study

The kinetic study of the DPA, luminol and PCM-CL systems was performed in a static mode in which all the experimental variables remained constant and were not changed. Typical reaction curves (intensity vs. time) for luminol (1.0×10^{-5} M in KOH, 1.0×10^{-3} M) CL reaction mechanism in the proximity of silver III complex (DPA 4.7×10^{-6} M) in KOH (8.0×10^{-3} M) enhanced by PCM 0.5 mg L^{-1} were examined and noted the kinetics of the reaction shown in Figure 3. As a result, when a PCM solution was introduced into the mixture of CL reagent and oxidant. The CL signals peak was seen at its maximum value within 0.6 seconds and get back to baseline after 1.2 seconds. The curve of kinetic reaction showed that the CL reaction was fast and extremely sensitive in the analysis of PCM in pharmaceutical samples, particularly, as DPA was employed as the oxidizing agent.
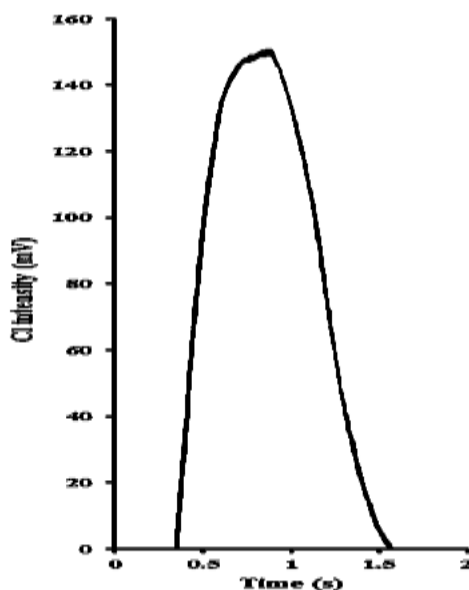
Figure 3. Kinetic Curve of DPA (4.7×10^{-6} M) in KOH (8.0×10^{-3} M), luminol (1.0×10^{-5} M) in KOH (1.0×10^{-3}M), PCM (0.5 mg L^{-1}) in 0.1 % v/v ethanol

3.2 Optimizations of various parameters
The variables used in the study were optimized to develop a fast and reliable analytical method for PCM analysis. The main variables include luminol, DPA, KOH, ethanol concentrations, and physical variables including sample volume, PMT voltage and flow rate. A standard solution of 0.5 mg L–1 PCM was used for optimization studies, and all were performed in triplicate.

3.3 Luminol effect as a CL reagent
Amongst the most employed reagents for CL oxidation under alkaline condition is luminol. The influence of luminol on Cl signal was studied in the range of 1.0×10^{-7} M to 5×10^{-5} M. An increase was seen in CL intensity up to 1.0×10^{-5} M with increase of luminol concentration when PCM was injected. Beyond this level, Cl signal decreased. Hence, 1.0×10^{-5} M of luminol concentration was selected as an optimum value and it was used throughout the study depicted in Figure 4(a). The study was performed in basic condition and therefore, the luminol should be prepared in a favorable concentration of KOH. The effect of KOH on CL signal of the reaction was measured in the range of 2.0×10^{-4} M to 4.0×10^{-2} M. The CL signals increased with increase of KOH concentration up to 1.0×10^{-3} M. Above this concentration, a decrease was noticed in Cl intensity as shown in Figure 4(b). Therefore, 1.0×10^{-3} M of KOH was considered and selected as the optimal and used throughout the experiment.

3.4 Effect of DPA as an oxidant
A highly effective oxidizing agent in a basic solution is a DPA with a standard reduction potential of 1.74 V. This is owing to its ability to carry out a two-electron oxidation in various situations. In this CL reaction, DPA was used as a proficient oxidant and had a significant impact on the CL signal. Hence, the concentration of DPA was further examined. The findings showed that the highest and most consistent CL intensity was achieved as the DPA concentration reached 4.7×10^{-6} M. Beyond this concentration, there was a decrease in CL signal owing to self-absorption, illustrated in Figure 4 (c). Thus, for later experiments, a DPA concentration of 4.7×10^{-6} M was selected as best. The stability of DPA was found in alkaline solution. Hence, the influence of different ranges of KOH was investigated ranging from 1.0×10^{-3} M to 3.0×10^{-2} M. The highest and most consistent CL signal was detected at 8.0×10^{-3} M concentration of KOH, as shown in Figure 4(d). Hence, 8.0×10^{-3} M concentration of KOH was picked out as the optimum and used in the research studies coming ahead.

3.5 Effect of ethanol as a carrier

To enhance the solubility of PCM, organic solvents, including ethanol, methanol, and acetonitrile, have been reported. Granberg et al. [40] reported the solubility of PCM in different solvents in g of PCM per kg of solvent at 30 °C. According to the report, 17.39, 371.61, 232.75, and 32.83 g of PCM can be dissolved in 1kg of water, methanol, ethanol, and acetonitrile, respectively. Therefore, ethanol was selected as a dissolving solvent for PCM keeping in view the solubility, toxicology and miscibility with water. In this study, absolute ethanol was employed for the preparation of PCM stock solution, and the influence of ethanol concentration was tested to increase its solubility. Ethanol was also utilized as a carrier for matrix matching. The range of ethanol concentration examined was from 0.025% to 1.0% (v/v). The CL intensity showed elevation in ethanol percentage up to 0.1% (v/v), as shown in Figure 4(e), due to the rise in solubility. Further increase inhibited the CL emission intensity. Based on this observation, a 0.1% (v/v) of ethanol was selected as the optimal concentration for this study.
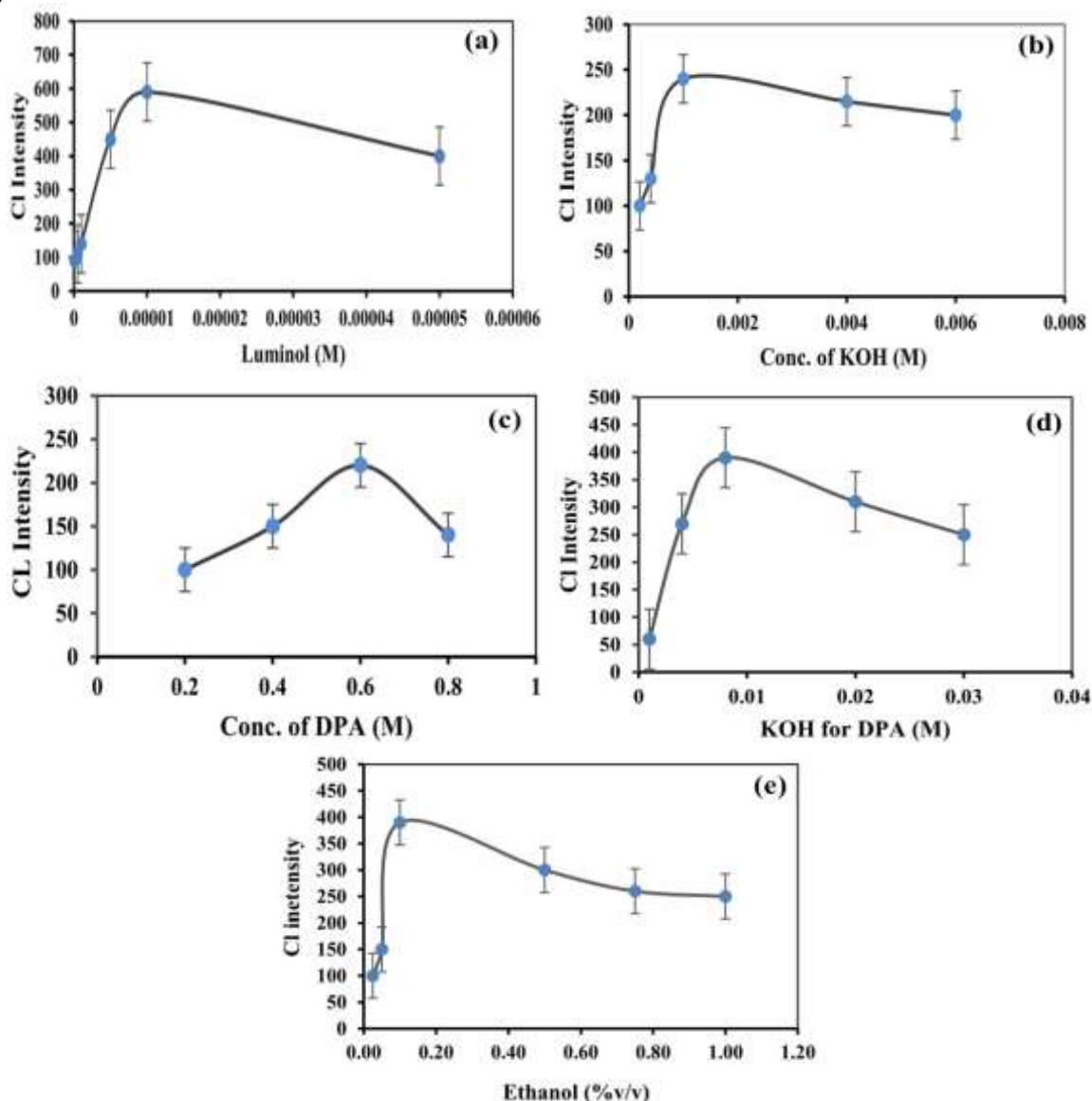

Figure 4. Effects of reagents (a), Luminol; (b), KOH for Luminol; (c), DPA; (d), KOH for DPA; (e), Ethanol concentration on Cl emission of PCM

3.6 Effect of physical variables

Physical parameters including flow rates, PMT voltage and injection volume were investigated as potential influencers on PCM CL intensity as displayed in Table 1. Kinetic studies revealed that the

proposed CL reaction was fast, so its effect on flow rate was examined from 1 to 6.0 mL min^{-1} and CL signal was elevated with increasing flow rates until reaching 5.0 mL/min as optimal. Sample injection loop volumes were evaluated between 45 to 260 µL, with CL intensity increasing linearly with sample loop volume up to 160 µL - therefore this volume was chosen as best. The emission of CL intensity in mV increased linearly as PMT voltage did, from 650 to 1000 V; however, 850 V was chosen as optimum owing to its high signal-to-noise ratio.

Table 1. Optimization of physical parameters for PCM ($n = 3$).

Physical Parameters	Range studied	Optimized value	RSD (%)
Sample Volume (µl)	45 – 260	160	0.9 – 2.9
Photomultiplier tube Voltage (V)	650 – 1000	850	1.6 – 3
Speed/ flow Rate (ml min^{-1})	1 – 6	5.0	1.2 – 2.6

3.7 Calibration Data

PCM concentration measured on the x-axis and CL intensity peak height measured on the y-axis presented a strong linear correlation within an optimal range of 0.075 – 0.75 mg L^{-1} ($R^2 = 0.9999$; $n = 4$). An equation of regression $y = 477.73x + 1.4471$ was obtained, where 'y' is CL signals measured in millivolts and 'x' is PCM level estimated in mg L^{-1}. The limit of detection and limit of quantification were found to be 7.0×10^{-4} mg L^{-1} and 2.4×10^{-3} mg L^{-1} with signal to noise ratio of 3 and 10 correspondingly. Additionally, relative standard deviation ranged between 0.3 – 1.1% within the studied range with injection rate at 120 hours^{-1}. Figure 5 displays chart recorder traces from PCM standard solutions (0.075–0.75 mg L^{-1}) while its standard plot can be seen in the inset.
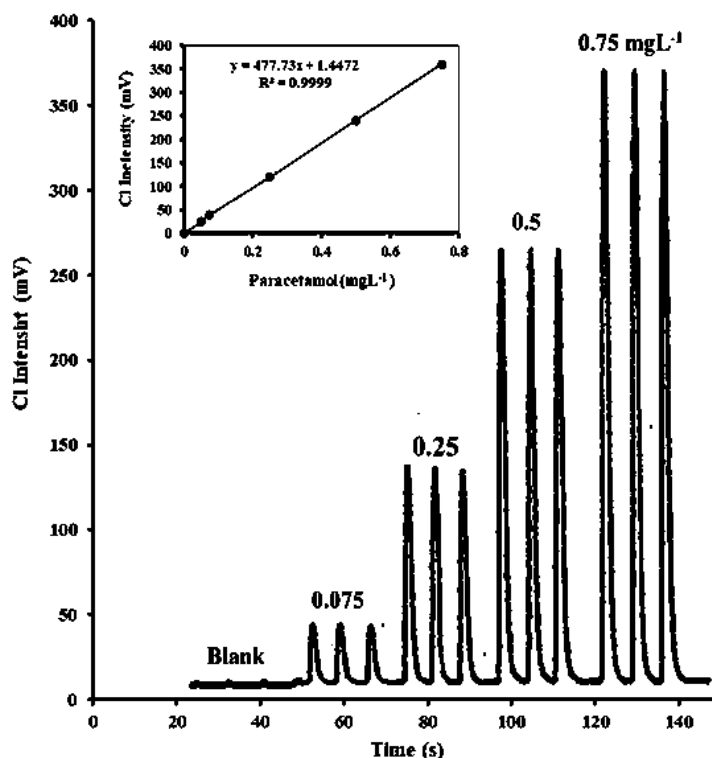

Figure 5. Output of chart recorder for PCM solutions injected in triplicate. Calibration curve is shown in the inset for PCM standard (0.075–0.75 mg L^{-1})

Table 2 presents a comparison between calibration data of current FI–CL and previously reported methods for detection of PCM in pharmaceutical and biological samples [15 - 26]. The values pointed out that the proposed FI–Cl method is better than most of the referenced methods. The advantages of the present study include ease of use, fast analysis time, higher sensitivity, a better linear dynamic

range, and a remarkably low detection limit. This newly suggested FI–Cl system is an excellent choice for the assessment of PCM in pharmaceutical tablets.

Table 2. Comparison of proposed FI–CL method for determination of PCM in tablets with previously reported methods

Technique	Calibration range (mg L^{-1})	LOD (mg L^{-1})	LOQ (mg L^{-1})	RSD (%)	Sample throughput h^{-1}	Reference
RP–HPLC	2.5–20	0.06	0.75	2.8	NR	[15]
RP–HPLC	100–600	0.01	0.03	NR	NR	[16]
UPLC	5.0–30.3	1.033	3.4	NR	NR	[17]
GC–MS	75–500	20	66	1.19	NR	[18]
Spec.	NR	0.03	0.08	0.709	NR	[19]
Spec.	1 – 20	0.520	0.875	0.93	NR	[20]
VM	5–150	0.02	0.065	NR	NR	[21]
VM	0.075–1511	0.025	0.089	NR	60	[22]
CE	9×10^{-5}–9.9×10^{-3}	8.4×10^{-4}	NR	2.9	NR	[23]
CE	15.116–2267.4	0.34	NR	1.4	NR	[24]
FI–Cl	3.7×10^{-3}–0.037	1.5×10^{-3}	NR	2.3	NR	[25]
FI–Cl	0.3–50.0	0.2	NR	1.1	90	[26]
FI–CL Luminol-DPA System	0.075–0.75	7×10^{-4}	2×10^{-3}	0.3	120	Present Study

RP–HPLC, Reverse Phase–High Performance Liquid Chromatography; UPLC, ultra–Performance Liquid Chromatography; GC-MS, Gas Chromatography–Mass Spectroscopy; Spec., Spectrometry; VM, Voltammetry; CE, Capillary Electrophoresis; FI–Cl, Flow Injection–Chemiluminescence; LOD, Limit of Detection; LOQ, Limit of Quantification; RSD, Relative Standard Deviation; NR, Not Reported

3.8 Interference study

Under optimized experimental conditions, we examined the impact of various organic interferences on the quantification of PCM (0.5 mg L^{-1}) and blank samples (Figure 6). This analysis involved injecting standard solutions of alien species (concentrations: 0.1, mg L^{-1}; 0.5, mg L^{-1} and 1, mg L^{-1}), which might be present as excipients into currently employed FI system and checking any interference which introduced relative errors of less than 5%. Sucrose, caffeine, glucose, fructose lactose and citric acid proved non-interfering with either PCM determination or blank indications; hence the suggested method proves to be suitable for PCM tablet.
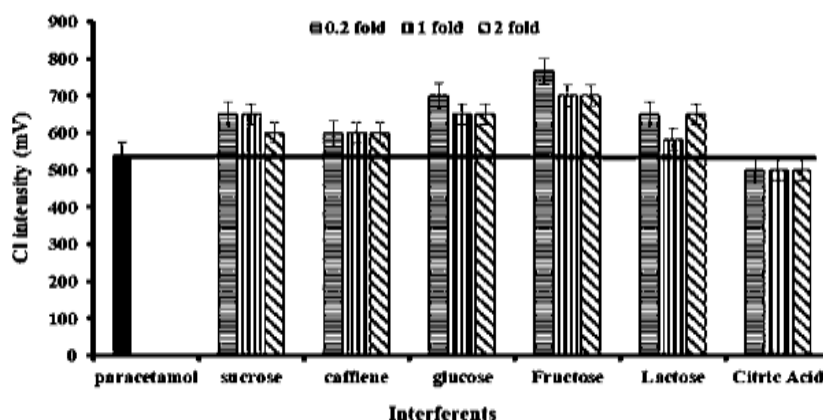

Figure 6. Interference study of organic species at 0.2, 1 and 2-fold of PCM 0.5 mg L^{-1}

3.9 Application of analytical system

The suggested method was utilized to detect the PCM level in pharmaceutical tablets and the consequences have been compared with a published FI–CL approach as illustrated in table 3. The findings were also analyzed with Student's t-test and variance ratio F-test which revealed that the suggested method was correct and précised. Furthermore, it was also confirmed with recovery tests. Several known fractions from a PCM stock solution were introduced into working standard mixtures of tablet dosages and tested with both suggested and existing FI–CL methods as shown in table 4. Recovery results for the present FI–CL procedure ranged from 89.1% to 108.3% with RSDs ranging from 0.2% to 2.4% while those for previously reported FI-CL methods ranged 87% to 109% with RSDs from 0.6% to 3.7%. All of these were performed in triplicate. Applying student's t-test, and F-test on the consequences of both methods showed that the difference if any were because of random error and not owing to systematic error with 95% confidence level.

Table 3. Analysis of PCM in pharmaceuticals tablets (Mean ± standard deviation, $n = 3$)

Samples	Labeled (mg tablet^{-1})	Present FI-CL Method Found (mg tablet^{-1})	Earlier FI-CL Method Found (mg tablet^{-1}) [26]
1.0	500	496.13 ±1.08	496.12 ±1.04
2.0	500	497.12 ±1.11	496.14 ±1.16
3.0	500	500.11 ±1.29	500.12±1.54

t-Tabulated (P = 0.05, v= 2): 4.302, Paired Student's t-test calculated: 0.378
F-Tabulated (P = 0.05, $v1$ and $v2$ = 2): 0.05, F-test calculated value: 0.08

Table 4. PCM tablets recovery results

Samples Matrix	Spiked (mgL^{-1})	Suggested FI-CL System			Published FI-CL System [26]		
		Found (mgL^{-1})	Recovery (%)	RSD (%) ($n = 3$)	Found (mgL^{-1})	Recovery (%)	RSD (%) ($n = 3$)
Tablet 1	0.000	0.097	–	2.4	0.099	–	3.7
	0.100	0.181	89.1	1.7	0.190	95.4	1.9
	0.250	0.369	104.7	1.6	0.371	106.2	1.7
	0.500	0.653	108.3	0.9	0.658	109.8	0.9
Tablet 2	0.000	0.099	–	3.1	0.099	–	3.8
	0.100	0.176	88.7	1.8	0.179	90	2.1
	0.250	0.365	104.6	0.8	0.366	104.8	1.0
	0.500	0.653	109	0.5	0.659	109.9	0.6
Tablet 3	0.000	0.100	–	1.2	0.101	–	3.4
	0.100	0.181	89.3	1.2	0.183	87.9	2.2
	0.250	0.375	106.6	0.3	0.355	99	1.1
	0.500	0.646	107.3	0.2	0.641	105.3	0.6

t-Tabulated (P = 0.05, v = 11): 2.201, Paired Student's t-test calculated: 0.272
F-Tabulated (P = 0.05, $v1$ and $v2$ = 11): 2.82, F-test calculated value: 1.005

3.10 Mechanism of Proposed CL Reaction

To determine the behavior of the chemiluminescence reaction, initial absorption spectra were recorded for reactants and analytes using a spectrophotometer (Model UV-1700 from Japan). Figure 7 clearly showed that PCM in 0.1% ethanol solution showed an absorption feature at 243.50 nm, as depicted in curve-a. The DPA solution 4.7×10^{-6} M in KOH solution 1.0×10^{-3} M and ethanol 0.1% v/v, revealed two peaks at 250 nm and 358 nm, as shown in curve-b. An additional solution of luminol at 1×10^{-5} M in 1×10^{-3} KOH with ethanol (1 mL each) produced three distinct peaks at 223.5, 300 and 349 nm as shown in curve-c. DPA-luminol mixtures produced redox reactions where DPA was the oxidant and luminol the reductant, leading to the respective disappearance and weakening of each

compound's absorption bands, indicated in curve-d, suggesting DPA as an oxidant and luminol as a reductant. Attributed to adding 30 mL of PCM solution per 3.0 mL to the mixture, as represented in curve-e, luminol absorption peak further diminished.
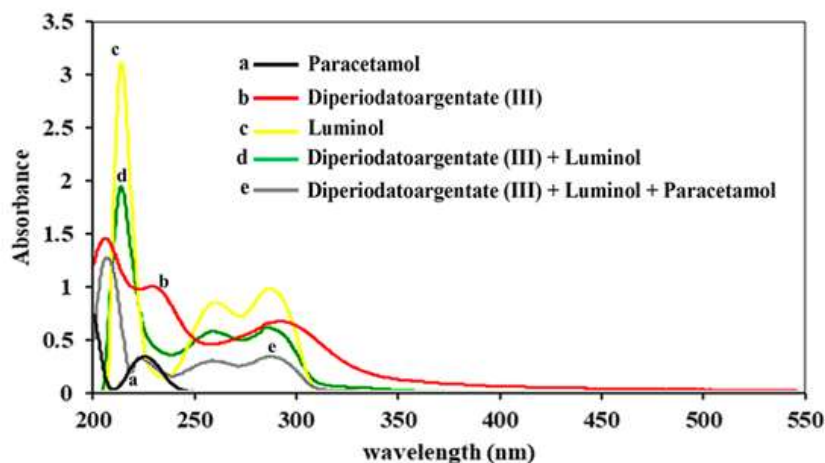

Figure 7. UV-Visible Curves (a) PCM (0.5 mg L^{-1}) in ethanol 0.1% v/v, (b) DPA (4.7×10^{-6} M) in KOH (8.0×10^{-3} M); (c) Luminol (1.0×10^{-5} M) in KOH (1.0×10^{-3} M); (d) Mixture of DPA and luminol 1.0 ml of each; (e) mixture of DPA, Luminol and PCM 1.0 ml of each.

Ambar et al. [35] used the CL reaction between DPA and luminol for the estimation of retinol (Vitamin A). Almost the same reaction has been followed by the suggested CL reaction i.e., luminol-DPA- PCM.

Figure 8 displays the transient peaks of luminol-DPA- PCM CL system running in flow mode. When ethanol solution (0.1% v/v) was propelled into all three channels luminol was introduced. No CL emission was seen, showing that luminol alone could not produce Cl signal as illustrated in Figure 8 (a). Likewise, luminol was derived into its specific channel and ethanol into other two channels, then DPA was inserted via injection valve, a background CL signal was seen without PCM presence shown in Figure 8 (b). Correspondingly, when all the reagents were pushed in their respective channels and PCM was introduced through injection valve, a strong Cl signal was seen, showing that flow injection can be used to develop a fast and précised system for the detection of PCM in pharmaceutical formulations as showed in Figure 8 (c).
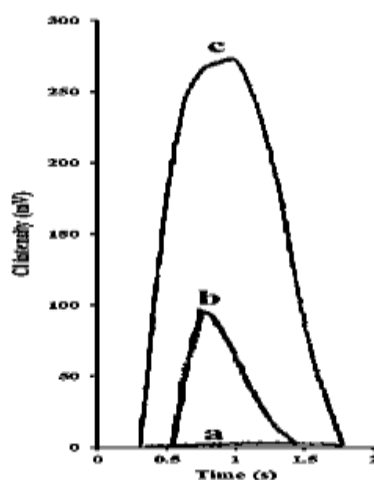

Figure 8. Cl intensity peaks of DPA-Luminol- PCM system in flow mode. (a), Luminol (1.0×10^{-5} M in KOH 1.0×10^{-3} M); (b), Luminol and DPA (4.7×10^{-6} M in KOH 8.0×10^{-3} M); (c), Luminol, DPA, and PCM (0.5 mgL^{-1} in ethanol 0.1% v/v).

The possible CL reaction mechanism is illustrated in Figure 9. Luminol and PCM are oxidized by DPA and generate different oxidized and reduced intermediate and final products. Silver either in zero or +1 oxidation state reacts with dissolved oxygen generating superoxide anion radicals which could further oxidize luminol and produce 3-aminophthalate in electronically excited state. This excited chemical species when it comes to ground state emits CL light at 425 nm. The intensity of the emitted CL correlates positively with the concentration of PCM.
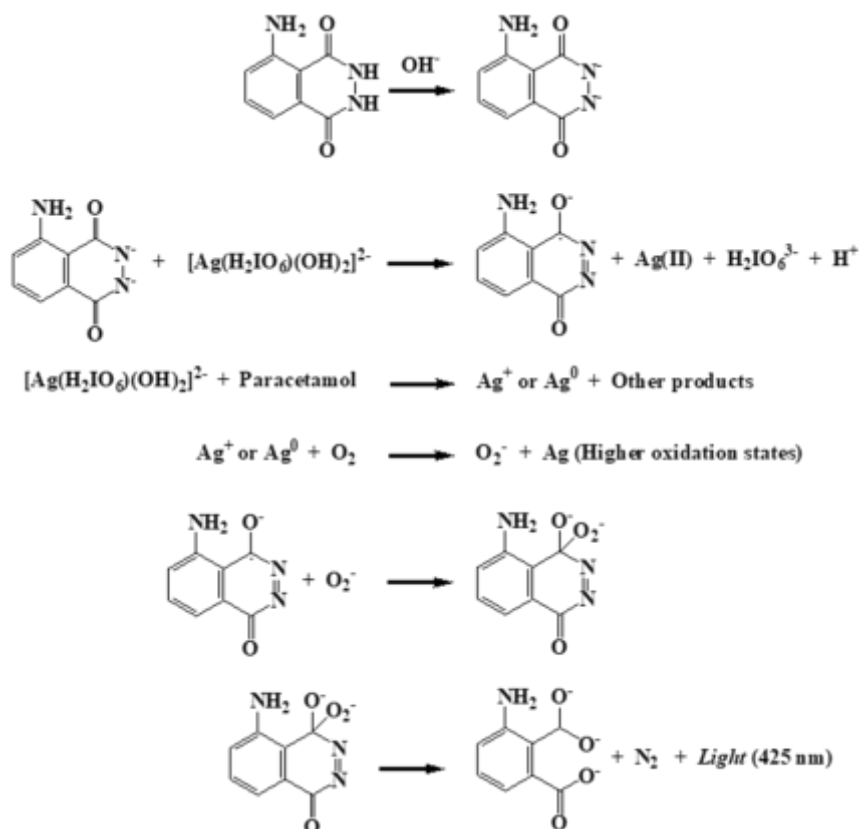

Figure 9. Possible reaction mechanism

4. Conclusion

In conclusion, this study focused on designing a significant and innovative FI–CL method specifically for PCM detection in pharmaceutical formulations. This approach depends on the oxidation reaction between DPA and luminol to produce a CL detection system which is sensitive, rapid, and broad-analytic ranged and with lower limits of detection compared to previously reported methods. The proposed method is distinguished by being completely free from interference from other substances, providing correct detections. Notably, the proposed method requires significantly less chemiluminescent reagent than previously reported methods, making it both cost-effective and environmentally sustainable. The proposed method has an increased sample throughput and reproducibility that are indicative of its suitability for high-throughput analysis in pharmaceutical quality control and environmental monitoring. This method has undergone extensive evaluation to prove its legitimacy as an alternative to existing methods. In addition, the suggested method was easily applied to PCM formulations.

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