

FLOW INJECTION ANALYSIS OF HYDROGEN PEROXIDE WITH PEROXYOXALATE CHEMILUMINESCENCE DETECTION

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This study reports a new, rapid and sensitive flow injection analysis (FIA) with peroxyoxalate chemiluminescence detection (PO-CL) for determination of hydrogen peroxide through merging zone principle. Di (N-Succinimidyl) oxalate was applied for the first time as peroxyoxalate chemiluminescence reagent. The CL was produced by the oxidation of Di (N-Succinimidyl) oxalate by hydrogen peroxide in the presence of a fluorescent compound, (9, 10 Bis phenyl ethynyl anthracene) and imidazole as a catalyst. Various parameters associated with this flow system were studied and essential optimizations were carried out. Calibration graph was constructed for determination of hydrogen peroxide in the range (0.02-0.34 mol.L⁻¹) with correlation coefficient (R²) (0.982). The method was applied successfully for the determination of hydrogen peroxide in commercial pharmaceutical products and in tap water.

KEYWORDS: Peroxyoxalate Chemiluminescence; Flow Injection Analysis; Hydrogen Peroxide; Di (N-Succinimidyl) oxalate.

1. INTRODUCTION

A peroxyoxalate chemiluminescence (PO-CL) method has been widely utilized in environmental, pharmaceutical and biomedical analyses owing to its high sensitivity and a need of simple instrumentation without a light source. In the PO-CL system, hydrogen peroxide (H₂O₂) reacts with oxalates or oxamides under co-existence of a fluorophore to produce an emission of light. The reaction is achieved by a chemically initiated electron exchange luminescence (CIEEL) mechanism via a high energy intermediate which forms a charge transfer complex with the co-existing fluorophore. Then the electron is transferred to the fluorophore, which transits to an excited state and returns to a ground state by emitting a photon (Wada, 2010).

Determination of H₂O₂ is of great importance in biochemistry, environmental fields and clinical control (Yeganeh, Amini et al. 2012). Many biological substances produce H₂O₂ in biochemical reactions catalyzed by various enzymes, so they can be determined indirectly by the determination of H₂O₂ (Yun-Xiang 1991).

Many methods have been reported for H₂O₂ determination including oxidation-reduction titrimetry (Izumi 2004; Brandhuber 2009), spectrophotometry (James 1977; Graf 1980; Iolanda 1998; Amar Kumar, 2002; Elnemma, 2004), fluorimetry (Yun-Xiang 1999), high performance liquid chromatography (Jinrong, 2005) and electrochemical (Ertas 2000; Parviz, 2010; Shokuhi, 2011).

A chemiluminescence method is commonly used in the determination of H₂O₂ because of its low detection limit and wide dynamic range that can be achieved with relatively simple instrumentation (Bowie, 1992).

Peroxyoxalate-chemiluminescence coupled with flow injection system is used for the detection of H₂O₂. Peroxyoxalate-chemiluminescence (PO-CL) is produced by the oxidation of bis(2,4,6-trichlorophenyl)oxalate by H₂O₂ in

presence of a fluorescent compound and Imidazole as a catalyst (Haruo, 1986; Nabi, 1996; Nozaki, 1999).

However, established systems for analytical purposes fall into one of a limited number of types, such as luminol, dimethylbisacridinium nitrate (lucigenin) and bis-(2,4,6-trichlorophenyl) oxalate (TCPO). Furthermore, these systems lack selectivity due to the possible interferences from catalyzing actions of many metal ions (Co(II), Cu(II), Fe(III), Cr(III) and Ni(II) for luminol-H₂O₂ system).

The purpose of this work is to develop a method for reliable quantification of H₂O₂ by peroxyoxalate chemiluminescence. The proposed CL-system can be applied for the determination of H₂O₂ in a natural water matrix and pharmaceutical products.

2. EXPERIMENTAL

2.1 Apparatus

The flow injection chemiluminescence system used in this work is shown in Figure 1. It consists of a peristaltic pump (Watson marlow 205u) 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 was 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 (30) μL were used to inject oxalate and fluorofour into the flowing carrier stream. A Y-shaped Perspex piece was used to mix the two streams of hydrogen peroxide and imidazol.

The streams 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 lightly blocked.

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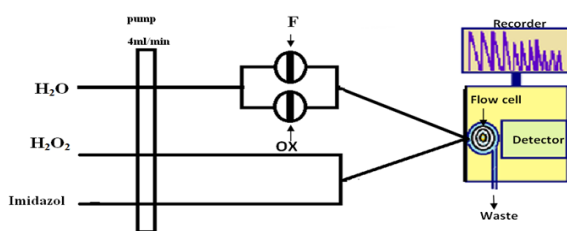


Figure 1. Schematic diagram of the FIA-POCL manifold used for the determination of hydrogen peroxide.

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

2.2 Reagents

All reagents were of analytical grade and distilled- deionized water (DDW) was used for preparation of their solutions. A stock solution (1×10^{-3} mole.L⁻¹ Di(N-Succinimidyl) oxalate (Sigma-Aldrich) solution was prepared by dissolving (0.0142) g of Di(N-Succinimidyl)oxalate in (50) mL ethyl acetate. Lower concentrations were prepared by dilutions of the stock solution with ethyl acetate in volumetric flasks. Stock (9, 10 Bis phenyl ethynyl anthracene) (Sigma-Aldrich) as fluorescent compound was prepared by dissolving (0.0189) g in (50) mL of ethyl acetate. Lower concentrations were prepared by dilutions of the stock solution with ethyl acetate in volumetric flasks. Imidazol (sigma aldrich) was prepared by dissolving (0.6808) g in (100) mL water. Lower concentrations were prepared by appropriate dilution with (DDW).

Hydrogen peroxide (0.5) mole.L⁻¹ was prepared daily by diluting (42.99) mL of hydrogen peroxide (labpak) (35) %, (1.13) g.mL⁻¹ with water in a (1.0) L volumetric flask. Working standard solutions were prepared by suitable dilution to obtain standard solutions for constructing calibration curves. The H₂O₂ solution was protected from light by placing it in brown bottle.

pH at range (5.8-8.0) were prepared by mixing sodium phosphate monobasic (BDH) and dibasic (BDH) solutions in the proportions indicated and the final volume adjusted to (200) mL with deionized water. The final pH is adjusted using a sensitive pH meter.

Stock solutions of each interfering species (0.1) mole.L⁻¹ were prepared in (100) mL water. Other solutions were prepared by serial dilutions of the stock solutions then adding of different amounts of each interference to a constant hydrogen peroxide concentration, and comparing the emission intensity with the sample with no interference. Hydrogen peroxide (20%) for pharmaceutical use was analysed by the proposed FIA-POCL method.

3. GENERAL PROCEDURE

The FIA manifold consisted a three-channel configuration where the oxalate and flouorfour solutions are incorporated to the carrier H₂O, (4) mL/min with the aid of the two rotary valves tied in parallel. A volume of (30) μL oxalate solution was injected manually in valve (1) and (30) μL of flouorfour solution was injected using valve (2). Both valves are turned and merged with a mixture of hydrogen peroxide and imidazol, with previous merging, in front of the detector. The emission light was detected and the peak height of the signal recorded as a CL- signal (mV). The presence of the hydrogen peroxide increased the PO-CL signal in a way that was proportional to its concentration.

4. RESULTS AND DISCUSSION

A series of experiments were conducted to establish optimum analytical variables in a flow injection system. The parameters optimized included reagent concentrations and manifold parameters. These were optimized for magnifying of hydrogen peroxide effect on the PO-CL signal.

4.1 Optimization of reagent concentrations

The effect of Di(N-succinimidyl)oxalate concentration on the chemiluminescence intensity was investigated over the range of (8×10^{-5} – 7×10^{-4}) mol.L⁻¹ fixing concentrations of hydrogen peroxide at (0.3) mol.L⁻¹, imidazol at (0.03) mol.L⁻¹ and [9,10 Bis phenyl ethynyl anthracene] at (5×10^{-6}) mol.L⁻¹. The optimum oxalate concentration was (5×10^{-4}) mol.L⁻¹ exhibited a maximum signal. Thus, this concentration is selected for further studies.

The influence of [9,10 Bis phenyl ethynyl anthracene] at different concentrations were tested at fixed concentrations of other reactants. The peak height increased gradually with [9,10 Bis phenyl ethynyl anthracene] concentration up to (5.0×10^{-5}) mol.L⁻¹. Thus, this concentration is selected for further studies.

The optimum concentration of imidazol was (3.0×10^{-2}) mol.L⁻¹ giving the maximum signal-to-blank ratio.

Effects of PH, is investigated over the range of (5.8-8.0), the optimum PH range (6.6-7.2) was agree with previously reported results.

Using de-ionized distilled water instead of buffer solution gave higher CL-intensity. Therefore, de-ionized distilled water was chosen for the further studies.

The type of solvent system employed in the PO-CL reaction was also studied to find out its influence on the CL intensity of the fluorophore. using a mixture of ethyl acetate /acetonitrile as a function of the type of organic solvent used for the preparation of Di(N-Succinimidyl) oxalate and [9,10 Bis phenyl ethynyl anthracene] solutions because the peroxyoxalate chemiluminescence reagents (oxalates) are insoluble in water. Moreover, they become unstable due to hydrolysis upon contact with water, and the hydrolyzed products lose their ability as chemiluminescence sensitizers. Highest CL -signal obtained by using ethyl acetate and two layers of solvents were observed in the sample cell as shown in Figure 2.

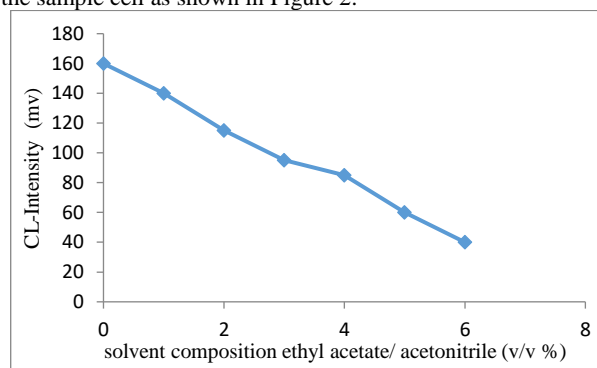


Figure 2. The effect of organic solvent composition on the PO-CL intensity

4.2 Physical, optimizations

Effects of length of mixing coil (0-40) cm was investigated on the PO-CL intensity. It was found that mixing did not affect the results as sensitivity and reproducibility considered.

The effect of flow rate in the range of (0.5-6) mL/min was examined using the optimized reactant concentrations. The signal increased with increasing flow rate from (0.5) mL/min to (5) mL/min, because the reaction rate is very fast and chemiluminescence will be observed immediately upon mixing.

At a flow rate higher than (5) mL/min, the reactants leaving the flow cell and CL were observed outside the detector optical path. Therefore, (4) mL/min was selected as the best flow rate.

5. CALIBRATION GRAPH

Under optimum experimental conditions in Table 1, the calibration graph of the relative PO-CL intensity is represented by peak height (mV) against hydrogen peroxide concentration ($\mu\text{g.mL}^{-1}$) as shown in Figure 3. Statistical treatments of the calibration results, including linear ranges, limits of detection, calibration equation and coefficient of correlation for hydrogen peroxide are shown in Table 2.

To determine the accuracy and precision of the proposed method, four replicate determinations were made on the three different concentrations of standard hydrogen peroxide 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 3.

Table 1. Summary of optimum chemical and physical conditions for determination of hydrogen peroxide by PO-CL.

Parameters	Optimum value
Di(N-Succinimidyl)oxalate	$5 \times 10^{-4} \text{ mol.L}^{-1}$
[9,10 Bis phenyl ethynyl anthracene]	$5 \times 10^{-5} \text{ mol.L}^{-1}$
imidazol	$3 \times 10^{-2} \text{ mol.L}^{-1}$
pH	6.6-7.2
solvent composition	Ethyl acetate
Flow-rate	4 ml/min
Mixing coil	0 cm

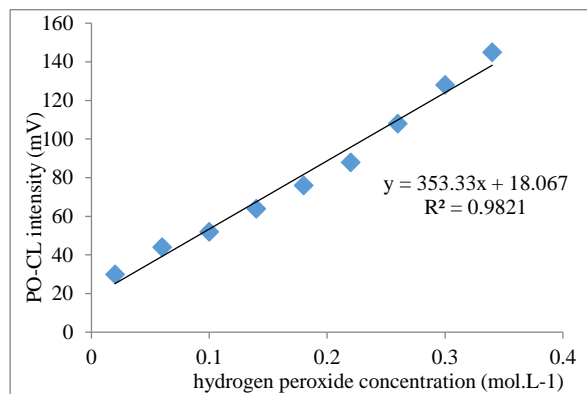


Figure 3. Calibration graph for the determination of hydrogen peroxide

Table 2. Analytical data for determination of hydrogen peroxide

Compound	Linear rang (mol.L^{-1})	Correlation coefficient	Linear regression equation	Detection limit (mol.L^{-1})
hydrogen peroxide	0.02-0.34	0.982	$Y = 353.3x + 18.06$	0.01

Table 3. Accuracy and precision of the present method

Compound	Conc. of hydrogen peroxide (mol.L^{-1})	Mean (mv)	E (%)	SD	RSD%
hydrogen peroxide	0.1	51.0	-3.7	1.91	4.5
	0.22	92	-3.86	2	2.1
	0.3	143	-3.6	1.15	0.8

6. INTERFERENCES

Interferences of foreign substances in the determination of H_2O_2 were studied by analysing standard solutions of hydrogen peroxide (0.1 mol.L^{-1}) to which increasing amounts of interfering ions were added. The salt concentrations were 100 times higher than the limiting concentration specified in drinking water. The results are summarized in Table 4.

7. APPLICATION

The procedure was applied successfully for the determination of hydrogen peroxide in commercial pharmaceutical products. For the aim of comparison, the samples were also analyzed by the reference method (titration with potassium permanganate)(Brandhuber and Korshin 2009).

The results are summarized in Table (5). 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 ($t\text{-calculated}=1.49 < t\text{-table}=2.75$ and $F\text{-calculated}= 3.80 < F\text{-table} = 5.05$ with a confidence limit of 95%).

The accuracy of the proposed method was checked with a recovery R (%) of various amounts of Hydrogen peroxide added to Hydrogen peroxide for pharmaceutical use. The results of the study are compiled in Table 6 which shows good accuracy.

8. SUGGESTED MECHANISM

In this work, it is the first time that Di (N-Succinimidyl) oxalate was reported as a reagent in peroxyoxalate chemiluminescence system. The chemiluminescence reaction between Di(N-Succinimidyl)oxalate solution and H_2O_2 produces a strong CL signal in the presence of 9,10 Bis phenyl ethynyl anthracene solution as fluorophore and imidazol as a catalyset as shown scheme 1.

Hydrogen peroxide reacted with Di (N-Succinimidyl)oxalate to produce 1,2 dioxetanedione, a high-energy intermediate.

A modified mechanism was proposed involving the transfer of an electron from the fluorophore to the reactive intermediate. The emission of light is thought to result from the annihilation of the fluorescer radical cation with the carbon dioxide radical anion formed when the 1,2-dioxetanedione decomposes. This process is called chemically-induced electron exchange luminescence (CIEEL).(Odile,1961 Baeyens,1998)

In the absence of imidazol, only a relative low CL was observed, which is mainly caused by Di(N-Succinimidyl) oxalate solution and hydrogen peroxide reaction.

9. CONCLUSION

In this investigation, we propose for the first time the application of the PO-CL system using Di (N-Succinimidyl) oxalate for the determination of hydrogen peroxide. The proposed CL detection is coupled to FIA manifold, which allows the application of the PO-reaction in organic solvent to prevent the decomposition of Di (N-Succinimidyl) oxalate. Di (N-Succinimidyl) oxalate might also undergo decomposition in solvents containing or miscible with water even though Di (N-Succinimidyl) oxalate is much more stable in ethyl acetate because ethyl acetate is immiscible with water.

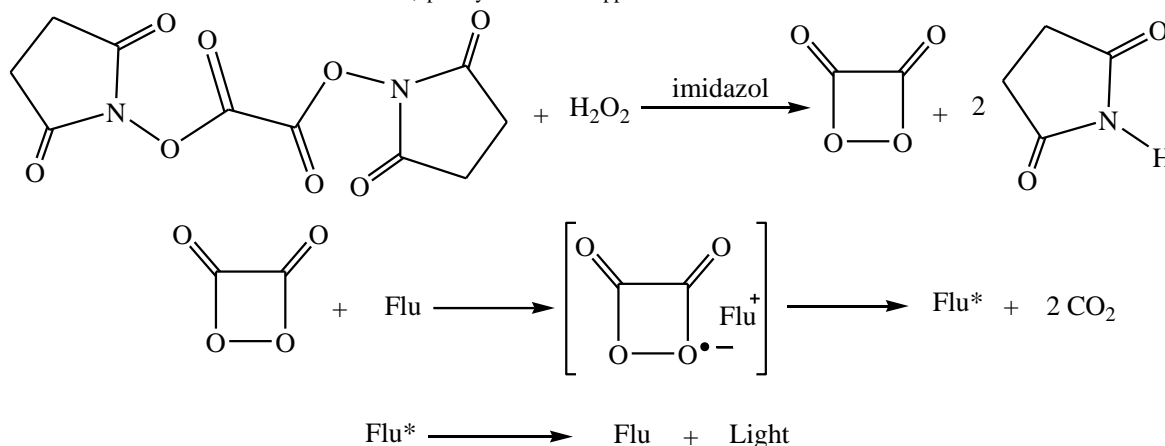
In relation to the above mentioned methods for the determination of H_2O_2 based on CL detection present suitable linear ranges and good limits of detection, but the proposed method is easier to implement for routine analysis in pharmaceutical control using cheaper and simple equipment. In addition the reaction occurs in organic layer that the foreign ions remain in aqueous layer leads to decreases the possibility of interference caused by common foreign species.

Compared with the direct chemiluminescence system utilizing luminol, peroxyoxalate chemiluminescence has a number of advantages.

First, it is more efficient and sensitive due to relatively higher ϕ_{CL} , and it is not susceptible to metal ion catalysis or effects of oxygen. Second, in addition to the detection of the oxidant which is the most applications of direct chemiluminescence reaction, peroxyoxalate

chemiluminescence can also detect other fluorophore-related species. Thus peroxyoxalate chemiluminescence has wider application scope.

Third, different from the alkaline requirement of direct chemiluminescence reaction, peroxyoxalate chemiluminescence reaction can be carried out at pH (7), the optimal pH for most biological process. Therefore, peroxyoxalate chemiluminescence is suitable for the biosensor application.



Scheme 1.

Table 4. Interference of foreign substances in the determination of H_2O_2 by PO-CL

Interference	Concentration H_2O_2 (mol.L ⁻¹)	Maximum concentration of interference ($\mu\text{g.ml}^{-1}$)	Recovery (%)
Br ⁻	0.1	1000	96
SO ₄ ⁻²	0.1	2500	100
NO ₃ ⁻¹	0.1	2000	99
HPO ₄ ⁻²	0.1	2000	100
Fe ⁺³	0.1	500	96
Ag ⁺¹	0.1	2000	100
K ⁺¹	0.1	1000	100
Na ⁺¹	0.1	5000	100

Table 5. Results of analysis of commercial drug formulations containing hydrogen peroxide by the proposed method.

Trade name	Labeled amount (mol.L ⁻¹)	Proposed method (mol.L ⁻¹)	Reference method (mol.L ⁻¹)	E (%)
Hydrogen peroxide for pharmaceutical use 20%	5.88	6.12	6.15	0.487

Table 6. Recovery experiments for hydrogen peroxide added to sample solutions.

Trade name	Initially present (mol.L ⁻¹)	Added (mol.L ⁻¹)	Found (mol.L ⁻¹)	Recovery (%)
Hydrogen peroxide for pharmaceutical use	0.1	0.07	0.175	102.9
		0.1	0.209	104.5
		0.15	0.254	101.06
Tap water	0.0	0.03	0.032	106
		0.07	0.073	104
		0.1	0.107	107
		0.15	0.158	105

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شیکردنه وهی کوتانی رۆبشتو له بو ههژمارکردنی هیدروجین بیروکساید به به کارهینانی بریسکهی کیمیاوی

کورتیا لیکولینی:

له م پرۆهیه دا ریگایه کی نوئ پيشانده دزیت كه فیرا و زور ورده له بو ژمارکردنی هیدروجین بیروکساید. ریگاکه به ننده له سه ر ژرانی رۆبشتو به به کارهینانی بریسکهی کیمیاوی له سه ر بناغه ی تیکه ل کردنی كه ناله كان. له م پرۆهیه دا یه كه م جار كه ره سه ته ی کیمیاوی (دای-ن- سکسینمایل اوکزالیته) به کار ده پنیته وهك ناسه ره وه له بریسکهی کیمیاوی دروست کراوه. بریسکه که دروست ده بیت به هوی ئوکساندنی (دای-ن- سکسینمایل اوکزالیته) به یارمه تی هیدروجین بیروکساید به بونی كه ره سه ته ی فلورسیننی (9 و 10-بس فنیل ایپانیل انتراسین) و امیدازول وهك یارمه تیده ری کارلیك. شرت و مه رجین پیۆانه ی له بو کارلیك به روه ست کراوه و گه نکه شه کردنی چونه ته ی به رپۆه چونی کارلیك. نه خشه ی پیوانه ی مه وداکه ی له به یینی (0.02-0.34 مول. لتر-1) له گه ل هاوکولکی به راوردگردن (0.982). ریگاکه به سه رکوتوی ئه نجام درا له بو هه ژمارکردنی هیدروجین بیروکساید له ناو كه ره سه تی ده رمانسازي و هه روه ها له ناو ئاود.

تقنية التدفق الحفني الجرياني مع البريق الكيمياء لتقدير بيروكسيد الهيدروجين

خلاصة البحث:

في هذا البحث يعرض طريقة جديدة، سريعة وحساسة لتقدير بيروكسيد الهيدروجين. الطريقة تعتمد على التدفق الجرياني والبريق الكيمياء مع تطبيق مبدأ دمج المناطق. في هذا البحث لأول مرة يعرض المركب ثنائي، سكسين – اميديتيل اوکزالیته ككاشف في البريق الكيمياء الذي يعتمد على البيروكسي – اوکزالیته. البريق الكيمياء ينتج من اكسدة المركب ثنائي، سكسين – اميديتيل اوکزالیته بواسطة بيروكسيد الهيدروجين بوجود المركب الفلورنسي (9، 10-ثنائي- فنیل ایپانیل انتراسین) و امیدازول كمحفز. تم ضبط الظروف المثلى للتفاعل ومناقشة آلية التفاعل. المنحني القياسي تتراوح بين 0.020-0.34 مول/لتر مع معامل الارتباط 0.982. الطريقة طبقت بنجاح لتقدير بيروكسيد الهيدروجين في المنتجات الصيدلانية وفي ماء الصنبور.