A Novel Chemiluminescence Assay of Ethylene Glycol in Antifreeze Samples using FIA with Merging Zone Principle

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Authors' contributions

This work was carried out in collaboration between authors. Author HSJ designed the study, managed the analyses of study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Author ATF managed the literature searches and approve the draft of the manuscript. The authors read and approved the final manuscript.

Research Article

ABSTRACT

Aims: Chemiluminescence determination of ethylene glycol.
Place and Duration of Study: Samples: Antifreeze samples purchased from markets (Erbil, Kurdistan region, Iraq), between April 2011 and August 2011.
Methodology: A novel chemiluminescence (CL) reaction was based on the oxidizing reaction of luminol by in situ generated bromine in alkaline medium. The CL intensity could be inhibited in the presence of ethylene glycol (EG). A new CL method was developed for the determination of EG by coupling with flow injection analysis (FIA) with merging zone principle. Because of the toxic and corrosive nature, difficult handling and high reactivity, which results in highly exothermic and non-selective reactions of bromine, the bromine (Br_2) will be generated in situ via the oxidation of hydrobromic acid (HBr) with hydrogen peroxide (H_2O_2).
Results: The relative CL intensity was proportional to the concentration of EG in the range of 35–440 µg/ml with the detection limit of 32 µg/ml and correlation coefficient of 0.9979. The relative standard deviation was 0.82% for 200 µg/ml EG.
Conclusion: The proposed method was successfully applied to the indirect determination of EG at the level of µg/ml in antifreeze samples. The error varied from –4.88% to 2.76%. A possible mechanism of the CL reaction was discussed by relating to the CL kinetic characteristics.

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1. INTRODUCTION

The use of molecular bromine as a reagent has several drawbacks arising out of its toxic and corrosive nature, difficult handling and its high reactivity, which results in highly exothermic and non-selective reactions. Additional problems arise from using chlorinated solvents and the release of corrosive HBr as a by-product. Alternative brominating reagents such as N-bromosuccinimide [1,2] and pyridinium or tetraalkylammoniumtribromides [3] make for easier handling and result in improved selectivity, but are unfortunately limited by their low atom efficiency and the need to remove the reagent's residue. Also, molecular bromine is required for their preparation. Oxidative bromination has potential in developing a sustainable and ecologically more acceptable procedure by the *in situ* preparation of an active brominating species via the oxidation of bromide using a suitable oxidant[4,5]. A diluted aqueous solution of hydrogen peroxide is a convenient, safe and environmentally favourable oxidizing agent that yields water as the effluent [5].

The wide applications of EG in both industrial and engine coolants have prompted extensive literature on its determination. Many methods have been reported for determination of EG such as chromatographic methods including gas chromatography (GC) [6,7], GC-MS [8,9], high-performance liquid chromatography (HPLC) [10,11], LC [12], TLC [13]. Also, spectrophotometric methods are among the most analytical methods used for EG determination in antifreeze [14–16]. Other reported methods include amperometry [17] and fluorimetry [18,19].

Flow analysis techniques are well-established toolsfor the automation and miniaturization of analytical methodologies, providing advantages such as: increased sample throughput, high versatility, high robustness, new analytical improvements based on operating modes undernon-stationary conditions, decrease of the human exposure under hazardous chemical/ physical sample pretreatments, more environmentally friendly procedures obtained due to process downscaling and use of alternative detection systems with the concomitant simplification of the operating conditions [20].

Luminol (5-Amino-2,3-dihydro-1,4-phthalazinedione)is the most widely used CL reagent. The CL emission of luminol is based on its oxidation by various oxidizing agents in the alkaline medium [20-22].

Evmiridis [23] reported a method for periodate determination which combines the rapidity of flow-injection analysis and the sensitivity of chemiluminescence detection. It is based on the CL emission generated during oxidation of pyrogallol by periodate, and gives a relative standard deviation of 3% and a detection limit of 0.35 µg with the instrumentation used. The method has been applied to determination of ethylene glycol, with a detection limit of 0.5 µmole. The accuracy of the method is quite good when the ethylene glycol is oxidized in unbuffered solutions, and the interference due to formaldehyde produced can be halved by prior addition of an appropriate amount of iodate. A throughput of 15 samples/min is possible, and the method is suitable for automation and remote control.

The present paper describes the development of a novel chemiluminescence assay of ethylene glycol using flow injection chemiluminometric system with merging zone principle based on the CL reaction of luminol with *in situ* generated bromine. In this green reaction Br_2will be generated *in situ* via the oxidation of hydrobromic acid with hydrogen peroxide.

Through optimization of experimental conditions the final procedure will be established for determination of ethylene glycol in antifreeze samples. Compared with the previous works of Evmiridis, in the proposed flow injection method with merging zones technique, the analysis speed is distinctly improved; and the consumption of reagents and sample is greatly decreased, which is very important for samples not easy to acquire; above all, the proposed method can be applied to determining EG in antifreeze samples without interferences effect.

2. EXPERIMENTAL

2.1 Apparatus

The flow system used for the determination and CL detection of EG shown schematically in Fig. 1. Two Desagapl - Heidelberg, England peristaltic pumps (6 channels, variable speed) were used to drive the carrier and the reagent streams through the flow system. Each stream was pumped at a constant flow rate using PTFE tubing (0.8 mm i.d.). Two rotary valves (Rheodyne U.S.A.) with variable loops were used to inject the sample (EG solution) and reagent (luminol solution) into flowing carrier streams. At the entrance of the flow cell the reagent (excess bromine) and luminol solution are mixed to produce CL. The mixing position of the flow cell was considered on the detector inside the spectrophotometer (JENWAY, 6405 UV/Vis. Spectrophotometer) the light source of which was blocked.
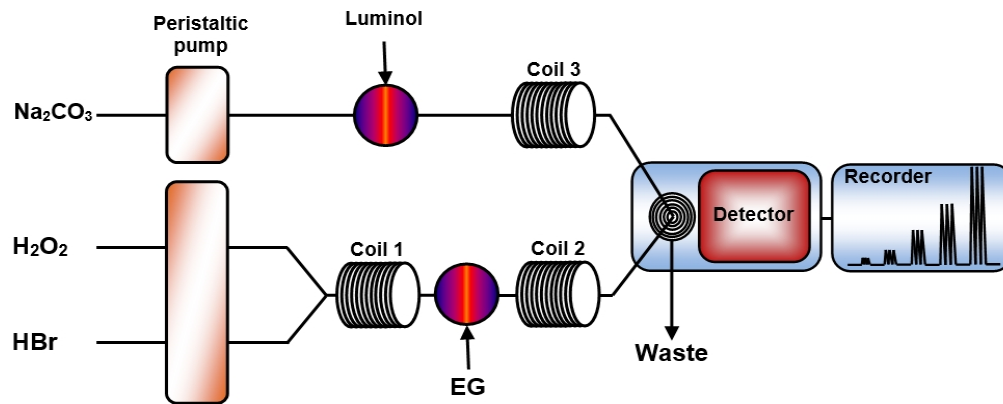

Fig. 1. The flow injection analysis manifold with merging zone principle used for CL determination of EG

The flow cell used in this work was made by winding the length of a glass tubing (0.8mm i.d) to form a coil with 60µl volume, in a way that the reagent and luminol solution are mixed exactly at the entrance of the cell. The signal was recorded by a Yokogawa Model 3021 recorder (Yokogawa, Japan). Peak height was measured for each signal. The analytical signal was calculated as sample output minus blank (change in CL intensity ΔI).

2.2 Reagents and Samples

Distilled water was used in all preparations. The reagents used were of analytical grades. Ethylene glycol (EG): A stock standard solution of ethylene glycol (1.0 mol/L) was prepared by diluting 56.3 ml of EG (99 %, BDH) to 1000 ml with distilled water. It was stored in a dark

bottle, and protected from daylight. The working standard solutions were prepared by suitable dilution.

Hydrobromic acid solution (1.0 mol/L): 56.18 ml of 48% (sp.gr. 1.5 g/ml) HBr (HW, Hopkin & Williams, England) was diluted to 500 ml with distilled water. Other solutions were prepared daily by serial dilution.

Hydrogen peroxide: A 1.0 mol/L hydrogen peroxide solution was prepared daily by diluting 6.69 ml of H_2O_2 (45% (v/v), GCC, 1.13 g/ml) in a 100ml volumetric flask with distilled water. The peroxide solution was standardized against standard 0.1M $KMnO_4$ [24,25]. This solution was protected from light and kept in brown bottle.

Sodium carbonate solution: 0.1 mol/L Na_2CO_3 solution was prepared by dissolving 10.599 g of Na_2CO_3 (Fluka – Garantie) in a small portion of DW and then completed to 1000 ml in a volumetric flask.

Luminol solution:1×10^{-3}mol/L solution was prepared by dissolving 0.1771 g of luminol (disodium salt of 5-amino-2,3-dihydro-1,4-phthalazinedione) (Surechem-LTD) in a little amount of 0.1 mol/L sodium carbonate solution, then completed to 1000 ml with the same solution in a volumetric flask. Other working solutions were prepared by suitable dilution with 0.1 mol/L sodium carbonate solution.

Sample preparation: 1.0 g of antifreeze was dissolved in water, and the resulting solution was diluted to the mark with water in a 100 ml volumetric flask. Then 10 ml of this solution was diluted 1000 fold [16]. 5.0 ml of this solution was analyzed for ethylene glycol, as described in preliminary procedure.

2.3 Preliminary Procedure

A flow injection analysis – chemiluminescence system (FIA–CL) with merging zone principle (Fig. 1) used in this work consists of two main streams which merges in a controlled manner. The first stream consists of *in situ* generated bromine (mixing of two other streams of hydrobromic acid with hydrogen peroxide) which 100 μL, 100 μg/ml EG (or distilled water in the case of a blank) was injected. While in the second stream a 100 μL portion of luminol solution was injected to the carrier sodium carbonate stream.
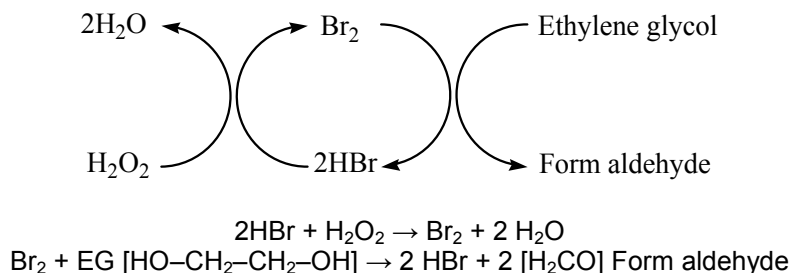
Luminol solution was injected after 12 sec of injection of EG according to merging zone principle so that the center of the both sample and reagent slugs match each other at the confluence point inside the flow cell which give rise to CL emission signal. The CL signal detected by spectrophotometer.

The concentration of the sample was quantified by the decrement of CL intensity, $\Delta I = I_0 - I_S$, where I_0 and I_S are CL signals in the absence and in the presence of EG, respectively. All measurements were performed at room temperature.

3. RESULTS AND DISCUSSION

The CL intensity reached a maximum in the absence of ethylene glycol (Br_2 react with luminol). If the concentration of Br_2 decreased by its reaction with EG (oxidation of ethylene glycol by bromine) the CL intensity decreases simultaneously. Therefore, the proposed CL

system was suitable for detecting ethylene glycol. The proposed mechanism of the reaction related to the method illustrated as Fig. 2 follows [4,26].

$$2H_2O \leftarrow \quad \rightarrow Br_2 \quad \longrightarrow Ethylene\ glycol$$

$$H_2O_2 \quad \longrightarrow 2HBr \leftarrow \quad \rightarrow Form\ aldehyde$$

$$2HBr + H_2O_2 \rightarrow Br_2 + 2\ H_2O$$
$$Br_2 + EG\ [HO–CH_2–CH_2–OH] \rightarrow 2\ HBr + 2\ [H_2CO]\ Form\ aldehyde$$

Fig. 2. In situ formation of bromine by oxidation of hydrobromic acid with hydrogen peroxide and oxidation of EG

To obtain the highest CL signal/noise, the flow rate of peristaltic pumps was also tested. The flow rate of pump 1 and 2 were all finally set at 1.0 and 2.0 ml/min as a suitable condition with superior sensitivity and reagent consumption. 100 microgram per millilitre ethylene glycol solution was used to optimize the experimental conditions.
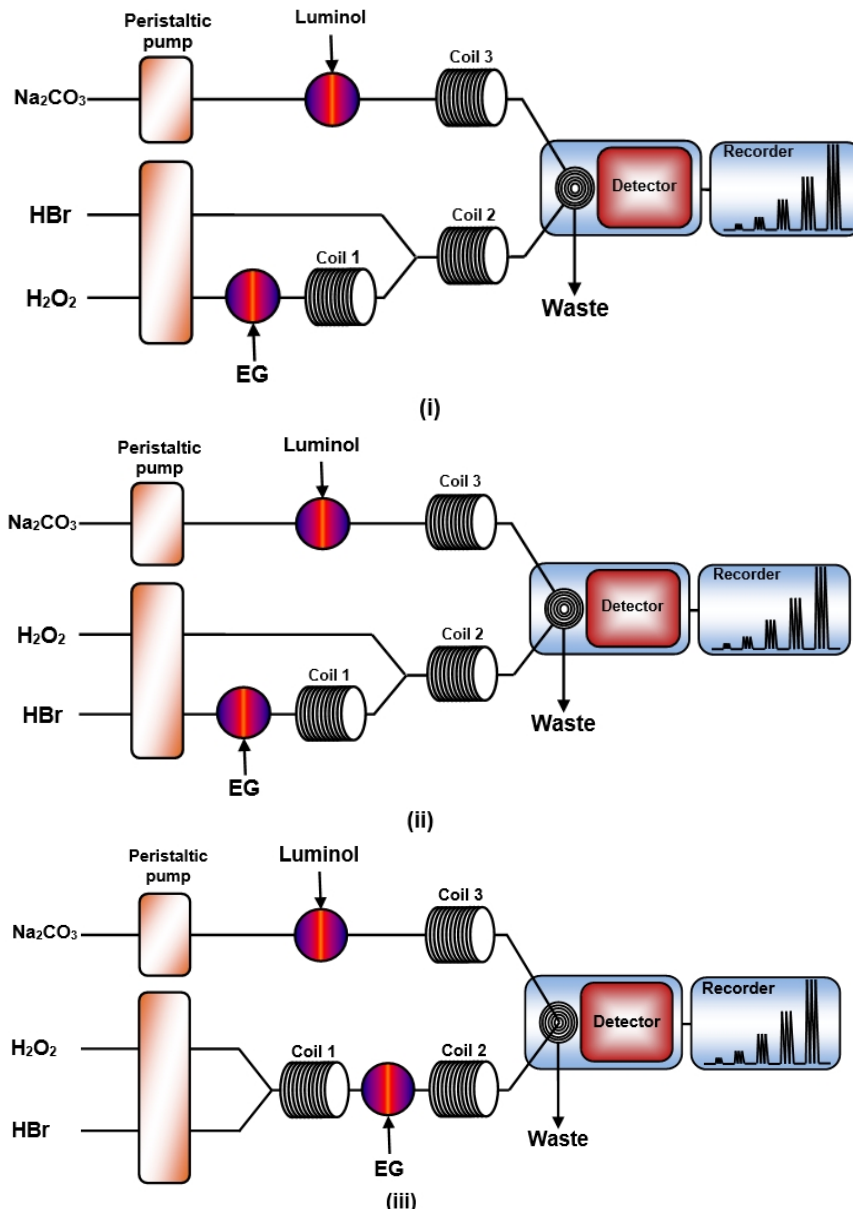
3.1 Optimization of Manifold Design

To establish the optimum conditions for the production of analytically useful CL emission, three FIA manifold designs were tested.

The first and second manifolds are depends on the injecting of EG solution into the hydrogen peroxide and hydrobromic acid streams respectively (Fig. 3, i and ii) and then formation of bromine to generate CL–light with luminol stream.

The difference between these two manifolds is that in the first manifold (Fig. 3, i) the sample injected into hydrogen peroxide line (i.e. oxidation of ethylene glycol by hydrogen peroxide). Then, the excess peroxide combined with hydrobromic acid solution to form bromine. While, in the second manifold (Fig. 3, ii) EG injected into hydrobromic acid solution (i.e. reaction of EG with HBr), then the unreacted HBr generate bromine with hydrogen peroxide stream.

The third manifold design was carried out by injection of the sample into bromine stream after combination of hydrobromic acid with hydrogen peroxide and *in situ* formation of bromine (oxidation of ethylene glycol by bromine) (Fig. 3, iii) then mixed with luminol stream and reaches the flow cell to generate CL – emission. The results shown that maximum CL intensity was obtained when EG solution was injected into *in situ* formed bromine stream which validates the reaction mechanism and then unreacted bromine was mixed with luminol line just before the detector (Fig. 3, iii).

Fig. 3. Schematic diagram of the manifold designs proposed for the determination of EG: i) (EG injection into H_2O_2) manifold. ii) (EG injection into HBr) manifold. ii) (EG injection into Br_2) manifold

3.2 Optimization of Chemical Parameters

Some parameters effecting on the flow system were examined and their optimum values are selected for operating the final method. Chemical parameters were varied while keeping the other flow injection variables constant. Optimizations were started using the following preliminary values; chemical parameters: EG (100 µg/ml), 0.1 mol/L HBr, 0.08 mol/L H_2O_2, 0.05 mol/L Na_2CO_3 and 6×10^{-5} mol/L luminol and physical parameters: flow rate of HBr, H_2O_2

and EG 1.0 ml/min, flow rate sodium carbonate 2.0 ml/min, coil 1: 50 cm, coil 2: 100 cm and coil 3: 10 cm, EG volume 100 µL and injection volume of luminol solution 100 µL at 25°C.

3.2.1 Effect of hydrobromic acid concentration

The influence of hydrobromic acid concentration on CL intensity was studied. As Fig. 4 shows, the CL emission produced during five seconds after injection of luminol solution to carrier stream. The analytical differences in signal resulted from CL- emission generated in the absence and presence of EG (100 µg/mL) (ΔI) was highest at a 0.15 mol/L concentration of HBr and then decreased rapidly with increase in the HBr concentration. A 0.15 mol/L concentration was therefore chosen for the reaction.
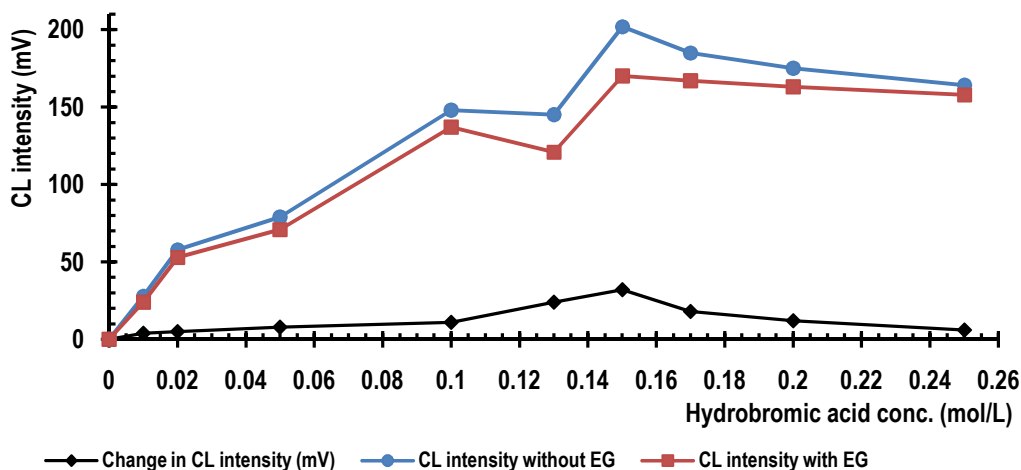

Fig. 4. Effect of hydrobromic acid concentration on the CL intensity.

3.2.2. Effect of hydrogen peroxide concentration

Keeping all other chemical and physical parameters constant, the effect of hydrogen peroxide concentration on the CL intensity was tested in the range of 0.005 – 0.16 mol/L, with three replicates of each concentration. The result in Fig. 5 shows that ΔI reaches a maximum value when the concentration of H_2O_2 is 0.06 mol/L, after that ΔI values remain nearly constant. Therefore 0.06 mol/L is chosen as optimum H_2O_2 concentration further works.

3.2.3. Effect of sodium carbonate concentration

The nature and concentration of the base used in the reaction had a very strong influence on the CL peak height. Several bases (0.05 mol/L), such as NaOH, KOH and Na_2CO_3 were tested in the carrier mixed with the luminol. Sodium carbonate was selected because it gave the best signal.

The effect of sodium carbonate concentration was studied. As shown in Fig.6, the difference in CL intensity was dramatically increased with the increase of Na_2CO_3 concentration and the highest CL signal was obtained in the 0.05 mol/L sodium carbonate, above which the ΔI of the system decrease. Therefore, 0.05 mol/L Na_2CO_3 solution was selected for subsequent experiments.
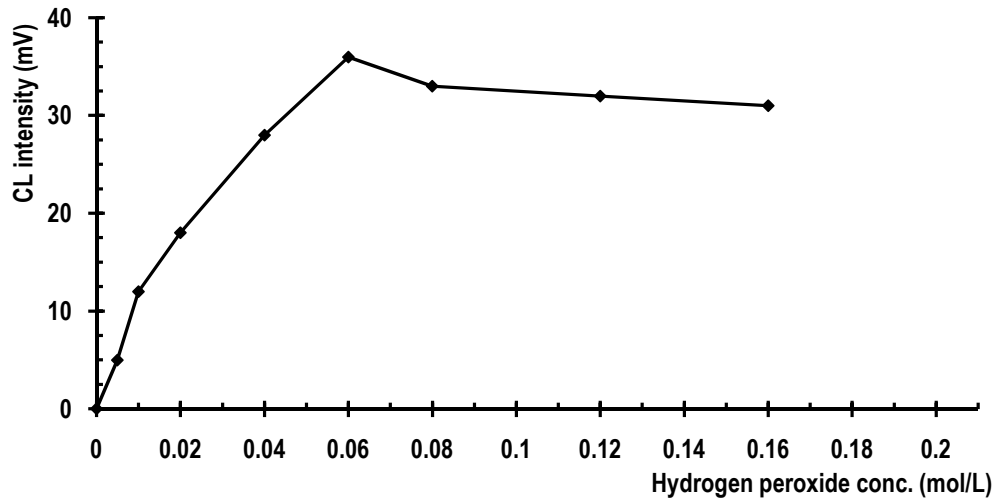
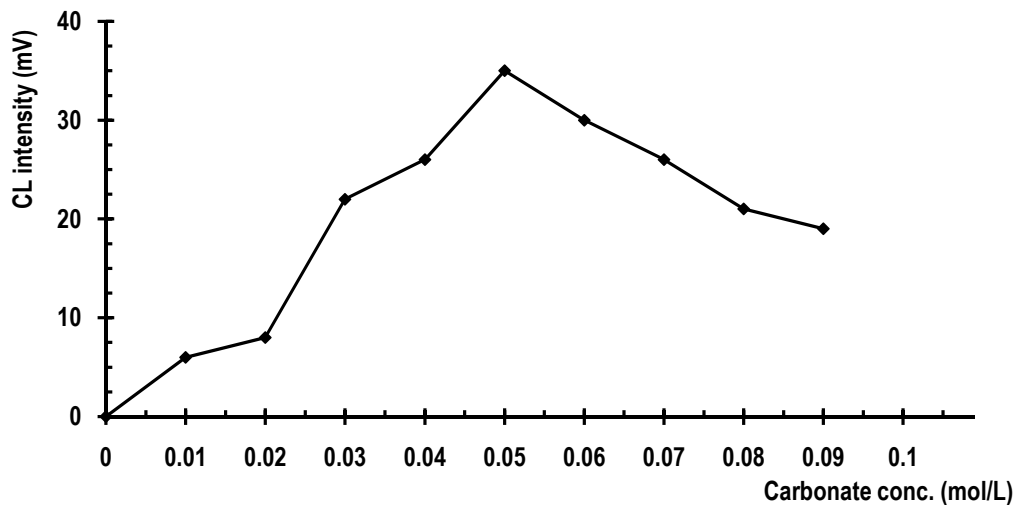**Fig. 5. Effect of hydrogen peroxide concentration on the CL intensity**

Fig. 6. Effect of sodium carbonate concentration on the CL intensity

3.2.4. Effect of luminol concentration

As the essential CL reagent for producing the chemiluminescent reaction, effects of luminol concentrations on the CL intensity were examined in the range of $1.0×10^{-5}$ – $12.0×10^{-5}$ mol/L. The CL intensity reached the maximum value at a luminol concentration of $4.0×10^{-5}$ mol/L (Fig.7). Above $4.0×10^{-5}$ mol/L the ΔI value decreased due to the self-quenching of luminol molecules [26–28]. Therefore, for the experiments, the concentration of luminol was maintained at the optimum value of $4.0×10^{-5}$ mol/L.
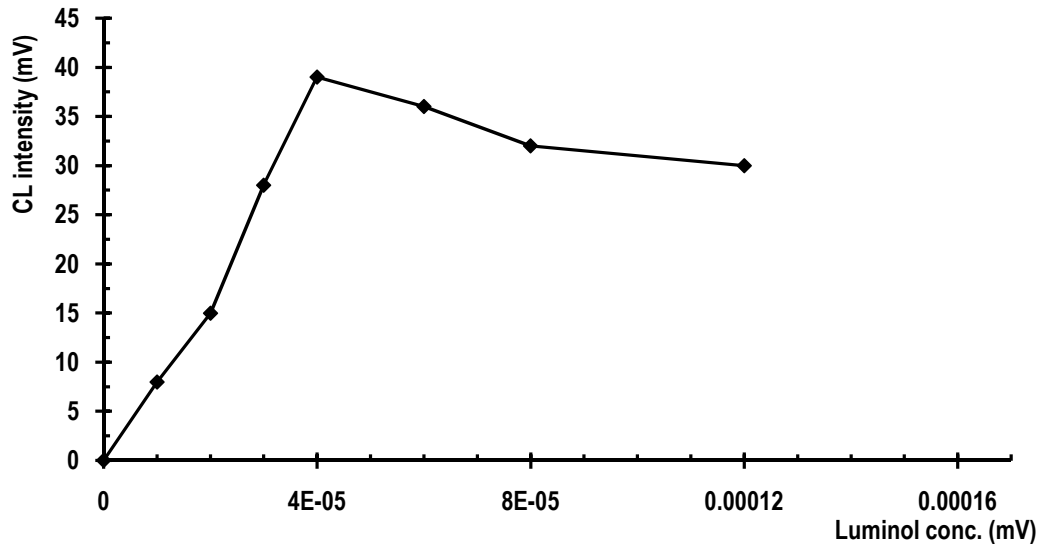
Fig. 7. Effect of luminol concentration on the CL intensity

3.3 Optimizations of Physical Parameters

3.3.1 Effect of flow rate

The flow rate is a critical parameter in FIA set-ups with CL detection because of the need to combine the excited product formation in the manifold with the maximum CL emission in the cell [29,30].

Flow rates of the solutions were tested in order to obtain a satisfactory CL peak using two peristaltic pumps. In this experiment, we controlled all of the solutions (HBr and H_2O_2 solutions) at the same flow rate, and changed the flow rate from 0.7 ml/min to 3.0 ml/min (except sodium carbonate line 2.0 ml/min) to study the effect of the flow rate on the CL intensity. The results in Fig. 8 showed that, the CL intensity (ΔI) continued to increase with increasing flow-rate up to 1.2 ml/min. A greater flow-rate could lower the ΔI value, because at high flow rates the reactants were reached to the detector at a shorter time which is not sufficient for completing the reaction. Therefore, a flow rate of 1.2 ml/min was selected.

The effect of sodium carbonate line flow rate was studied (flow rate of other lines controlled – 1.2 ml/min). As shown in Fig. 8 the highest emission was obtained using a 2.0 ml/min flow rate, probably due to need of fast mixing of excited product.

3.3.2 Effect of coil length

Effects of lengths of reaction coils 1, 2 and mixing coil 3 (Fig. 1) on the analytical signal were studied by keeping all other variables constant.

Coil 1 was used to mix hydrogen peroxide with hydrobromic acid to form Br_2. An increase in the length of this coil from 5.0 cm to 50 cm leads to increasing of ΔI value (Fig. 9) because of increasing of reaction time. On the other hand, instability of the formed Br_2 causes a reduction in signal obtained when longer coil is used [25]. Therefore, a 50 cm tubing length (coil 1) was chosen as suitable reaction tube for further experiments.

Effect of reaction coil 2 length was studied by varying the tubing length from 5.0 to 160 cm. The coil 2 is used to oxidize EG by Br_2. It was clear that the sensitivity increased with increasing in coil length up to 100 cm, further increase in tubing length led to the reduction of sensitivity. A 100 cm coil 2 length was therefore chosen, taking into account sensitivity of the procedure.

The effect of length of mixing coil 3 was studied in the range of 5.0–40 cm. Normal behaviour is observed in the curve, with an initial increase caused by the better mixing of the injected reagent (luminol) and flowing carrier (sodium carbonate), a maximum effect reached at 10 cm, and a decrease caused by the dispersion observed for longer reaction coils. Hence, a 10 cm mixing coil length was selected for further experiments.
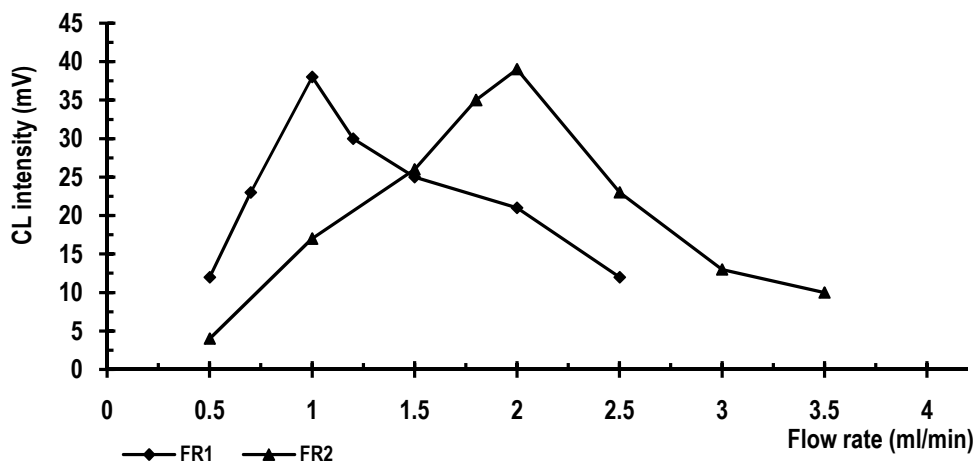

Fig. 8. Effect of flow rate on the CL intensity; FR 1: flow rate of all lines except luminol, FR 2: flow rate of carbonate–luminol line
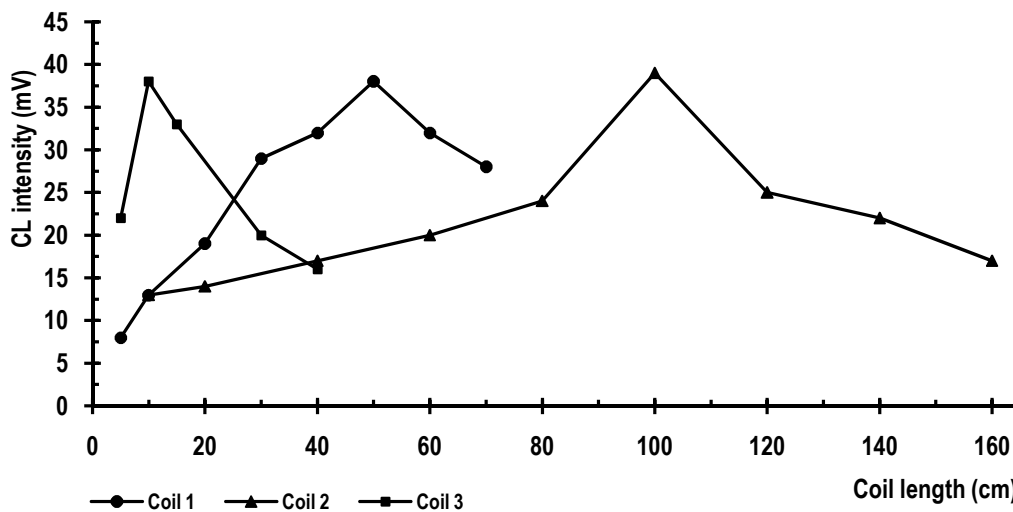

Fig. 9. Effect of coil length on the CL intensity; coil 1: reaction of HBr and H_2O_2, coil 2: reaction of Br_2 and EG, and coil 3: mixing of carbonate and luminol

3.3.3 Effect of luminol volume

The variation of CL emission with the injected reagent volume (luminol – pH about 10.5) in the 50 – 175 µl range was studied. The CL intensity increased with increasing the luminol volumes up to 125 µl (Fig.10); above this volume, the peak height was decrease because the mixing of the reagents was insufficient and peak broadening appeared. Therefore; a sample volume of 125 µL was selected for the procedure.
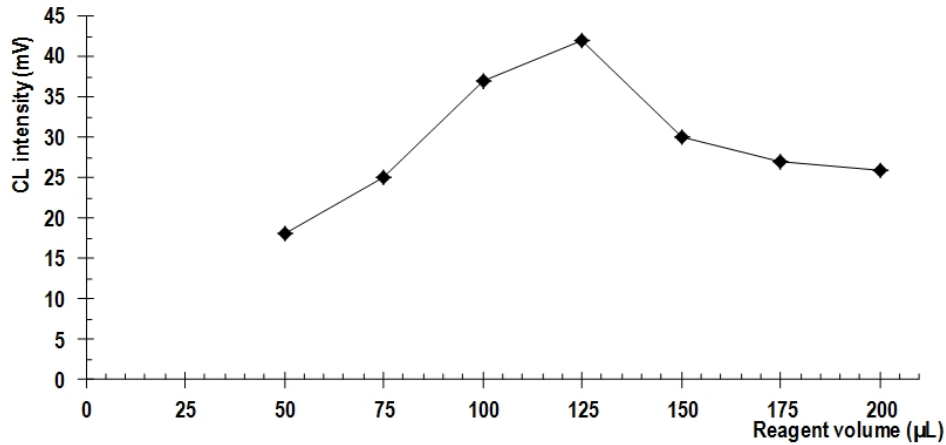

Fig. 10. Effect of luminol volume on the CL intensity

Optimum working conditions employed in this study for the determination of EG by proposed FI–CL method, are illustrated in Table 1.

Table 1. Optimum conditions for determination of EG

Parameter	Optimum value
Hydrobromic acid concentration	0.15 mol/L
Hydrogen peroxide concentration	0.06 mol/L
Sodium carbonate concentration	0.05 mol/L
Luminol concentration	0.00004 mol/L
Flow rate peristaltic pump 1	1.0 ml/min
Flow rate peristaltic pump 2	2.0 ml/min
Coil length: coil 1, coil 1 coil 1	50, 100, 10 cm
EG volume	100 µL
Reagent volume (luminol solution)	125 µL

4. CALIBRATION GRAPH

Under the optimum conditions described in Table 1 using the proposed FI-CL manifold in Fig 1, calibration graph was obtained for EG by plotting the graph of ΔI (change in CL intensity) vs. EG concentration (µg/ml). Three injections were used for each concentration. The CL intensity was decreased with increasing EG concentration and ΔI increased showing a linear range of 35 – 440 µg/mL with a correlation coefficient of 0.9979and the detection limit was 32 µg/mL. The statistics and performance characteristics are summarized in Table 2.

Table 2. Illustrates the statistical and optical treatment of calibration graph of EG

Analyte	Blank signal (mV)	Linear range after optimization (µg/ml)	Slope (b)	Intercept (a)	Correlation coefficient (R)	Detection limit (µg/ml)	Linear range before optimization (µg/ml)
Ethylene glycol	280	35 – 440	0.6894	-20.248	0.9979	32	100 – 400

The repeatability of the proposed method, expressed as relative standard deviation (R.S.D.%) of the peak height, for three measurements of two independent solutions containing 200 µg/ml and 345 µg/ml of standard EG, and included into the linear range were 0.82% and 0.05%, respectively. The error percentage (E%) for these solutions are shown in Table 3.

Table 3. Evaluation of accuracy and precision of the EG determination

Analyte	Analyte concentration (µg/ml)		E%	RSD %
	Standard solution	Calculation from proposed method		
EG	200	201.84	0.92	0.82
	345	345.18	0.05	0.05

5. INTERFERENCES STUDY

Influences of foreign species and components in commercial products of antifreeze solutions were investigated by analyzing a standard solution of EG of 180 µg/ml to which increasing amounts of interfering species were added.

The tolerable concentration ratios with respect to 180 µg/ml EG for interferences at the 5% level were over 5.0 for borate, phosphate, silicate: 2.5 for benzoic acid: 0.5 for sodium fluorescein (Table 4). Because the contents of coexisting substances and excipients in commercial antifreeze solutions are all lower than their tolerable concentrations after certain dilution, the proposed method could be used selectively to determine EG in antifreeze samples.

Table 4. Study of the effect of interferences on the CL determination of EG

Interfering species	Maximum Allowable Concentrations (µg/ml)	Ethylene glycol (µg/ml)		Error %	Tolerable Concentration Ratio[b]
		Added	Found[a]		
Borate $(BO_3)^{3-}$	900	180	181.68	-0.93	5.0
Phosphate $(PO_4)^{3-}$	900	180	178.78	0.68	5.0
Silicate $(SiO_4)^{4-}$	900	180	178.05	1.08	5.0
Sodium fluorescein	90	180	171.52	4.71	0.50
Benzoic acid	450	180	184.58	-2.54	2.5
Mixture of all		180	175.87	2.29	

[a]*Mean of three replicate analyses*
[b]*Tolerable Concentration Ratio with no interference [Conc. Interferent(µg/ml) / Conc. EG (µg/ml)]*

6. APPLICATIONS

Following the procedure described in section 2.3, the proposed method was applied to assay EG in antifreeze solutions. Six different samples were purchased from the local market and analysed. These antifreeze solutions contained a diverse amount of EG.

Mean error values ranging from – 4.88% to 2.76% were obtained (Table 5). The composition values reported by the supplier and those obtained by standard method [16,31] showed a good agreement with the results obtained by applying the present CL method, so proving its applicability. The results of FI–CL method were compared with that obtained by standard method employing t–test and F–test indicated that there was no significant difference between accuracy and precision of the two methods at 95% confidence level for five degrees of freedom ($t_{calculated}$= 0.95 <t_{table} = 2.57 and $F_{calculated}$ 0.52 <F_{table} 5.05 [32,33].

Table 5. Results of the determination of EG in antifreeze solutions using proposed FI–CL method

Trade name	Ethylene glycol [a]			E %
	Labeled amount (%)	Detectable amount (%)		
		Proposed method	Standard method [b]	
Prista Ready Antifreeze	42 %	43.14	42.13	2.40
Fuchs Antifreeze (Summer Coolant)	50 %	50.45	52.28	– 3.50
Deepen Cam SuyuAntifirizi	36 %	37.25	36.25	2.76
Sea Horse Antifreeze	50 %	49.11	51.63	– 4.88
Dynagel Plyn Do Chlodnic 2000	50 %	50.11	49.75	0.72
National Antifreeze	50 %	48.77	48.15	1.29

[a]*Average of five replication (n=5).*
[b]*Standard method (Iodometric titration)*

7. CONCLUSIONS

The potentials of FIA are pointed out to a great extend when unstable reagents have to be used. These reagents can be produced *in situ* in the FIA manifold, offering the advantages of simplicity, rapidity and eliminating the need for frequent standardization and storage under specific conditions.

Therefore; a new flow-injection CL method is developed for the determination of ethylene glycol in which bromine can be produced on-line by oxidation of bromide by peroxide. Ethylene glycol was determined indirectly, based on the decrease of bromine–luminol chemiluminescence. The system exhibits good stability, reproducibility and satisfactory detection limit. The sensitivity of proposed CL method is not less than that obtained for determination EG using spectrophotometric [15] method that give linear range between 40-200 µg/ml respectively. The proposed method is less time consuming (about 60 samples/min) comparing with some other spectrophotometric methods [34,35] that need about 30 and 20 minutes respectively for total analysis. In addition, some of them like GC-

MS [8,9] and HPLC [10,11] higher cost from the proposed method as an additional disadvantage of the proposed method. Therefore, the method can be applied to the determination of ethylene glycol in antifreeze samples. The proposed method offers the advantages of simplicity, rapidity, and good sensitivity for the determination of EG.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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