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Spectrophotometric - Flow Injection Analysis Determination of Hydrogen Peroxide Via Quenching of Bromine Absorbance

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Abstract: A proposed method is described for the determination of hydrogen peroxide in three pharmaceutical samples based on the in situ generation of bromine from the BrO_3^- - Br^--H_3O^+ reaction; the bromine is give a continuous absorbance measured by Ayah $3S_{BGR}$X3-3D solar cell CFIA microphotometer and then when injecting hydrogen peroxide, it will quenching the response because bromine molecules are converted to bromide ions. The linear range of 0.01-15mmol.L^{-1} along the correlation coefficient (r) of 0.9959, limit of detection (LOD) of 14.45ng/sample. A comparison was made between two methods: newly proposed method and the classical method using the standard addition procedure, via the paired t-test and there was no significant difference between the two methods at 95% confidence level.

Keywords: Hydrogen Peroxide, Spectrophotometer, Flow Injection Analysis.

INTRODUCTION

H_2O_2 (hydrogen peroxide) is an important chemical oxidant reagent play a role in various applications such as industries, laboratories, treatment of waste waters and pharmaceutical industry. It is a colorless, clear liquid that is present in different concentrations in aqueous solution. The effects of hydrogen peroxide may only cause toxicity from all routes of exposure.

At high concentrations levels cause damage of the skin, lungs and eyes. The principal product of hydrogen peroxide reactions is H_2O so it is preffered in many industries as oxidizer reagent.

One of the most important species (reactive oxygen) generated in the body is H_2O_2. Temporary whitening or bleaching of the skin can be caused by contact with hydrogen peroxide liquid[1-4].

The techniques for the determination of hydrogen peroxide can be classified into: spectrophotometric[5,6], colorimetric[7-9], high performance liquid chromatography[10] and electrochemically[11]. Many procedures based on chemiluminescence-flow injection[12-15] analysis was used for the determination of hydrogen peroxide such as using a new noble chemiluminscence and fluorescence cell[16] designed for the measured chemiluminescence and fluorescence energy transfer contained three inlet one for the donner molecule, another for the acceptor fluorophore molecules and third one for the carrier stream line. This research aims to develop a new method of estimating hydrogen peroxide using homemade Ayah $3S_{BGR}$X3-3D[17] coupled with continuous flow injection technology based on quenching of continuous absorbance response of bromine molecules which released from the reaction between Br^- with H_3O^+ in the presence of BrO_3^-.

CHEMICALS

Hydrogen peroxide solution, 100mmol.L^{-1} (BDH). Dilute 19.44mL of 35% H_2O_2 to 2L with distilled water. (Molarity of H_2O_2 fixed in H_2SO_4 medium (1:1) with $KMnO_4$ (0.1mol.L^{-1}) and standardized with 0.1mol.L^{-1} $Na_2C_2O_4$.

Potassium bromate solution, 100mmol.L^{-1} (BDH).Dissolve 8.3505g of $KBrO_3$ in 500mL of distilled water

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Potassium bromide solution, 0.01mol.L^{-1}(BDH). Dissolve 0.59501g of KBr in 500mL of distilled water.

Hydrochloric acid, 1mol.L^{-1}(BDH).Dilute 44.14mL of 35% HCl (sp.gr.1.18)with distilled water in a 500mL calibrated flask. Standardized with Na_2CO_3 solution

Nitric acid, 1mol.L^{-1}(BDH). Dilute 31.7mL of 70% HNO_3 (sp.gr.1.42) with distilled water in a 500mL calibrated flask. Standardized with Na_2CO_3 solution

Sulphuric acid, 1mol.L^{-1}(BDH). Dilute 27.8mL of 96% H_2SO_4 (sp.gr.1.84) with distilled water in a 500mL calibrated flask. Standardized with Na_2CO_3 solution

Acetic acid, 1mol.L^{-1}(BDH). Dilute 28.7mL of 99.5% CH_3COOH (sp.gr.1.05) with distilled water in a 500mL calibrated flask. Standardized with NaOH solution

APPARATUS

New system for microphotometric determination of hydrogen peroxide was used in this work via Ayah $3S_{BGR}$X3-3D solar cell CFIA microphotometer. The instrument uses three sources of light emitting diode (Blue 470nm, Green 525nm and Red 635nm) to cover up the visible region, with three solar cells as a detector ; each solar cell for a single source i.e three LEDs of the same wavelength as each set of three LEDs represent single source of radiation. Flow system consist of : peristaltic pump (ISMATEC, Switzerland, type ISM796), connection tubes, injection valve (model,V-450)(upchurch, scientific INC), Y-junction point and reaction coil. The readout of the system composed of X,Y-t potentiometric recorder(Kompenso Graph C-1032) Siemens, Germany.

METHODOLOGY

Fig.1 shows a flow injection system represented by schematic diagram used for the determination of hydrogen peroxide. Line 1 supplied a mixture solution from 0.3mmol.$L^{-1}BrO_3^-$-0.08mmol.$L^{-1}Br^-$(flow rate 1.6mL.min^{-1} for both lines) which meet with 0.3mmol.L^{-1} nitric acid solution at Y-point (junction point) to release of bromine (red-brown product,381nm, followed by equation no.1) and then to delay reaction 100 cm of coil to complete the formation of bromine which give the continuous absorbance measured by Ayah$3S_{BGR}$ X3-3D-solar cell by using irradiation source blue light emitting diode (470nm). After that hydrogen peroxide injected through the sample loop (85µL) into a stream of bromine and converted it to bromide ion which the solution becomes colorless again (quenched a continuous absorbance, equation no.2) and recorded the signal obtained.

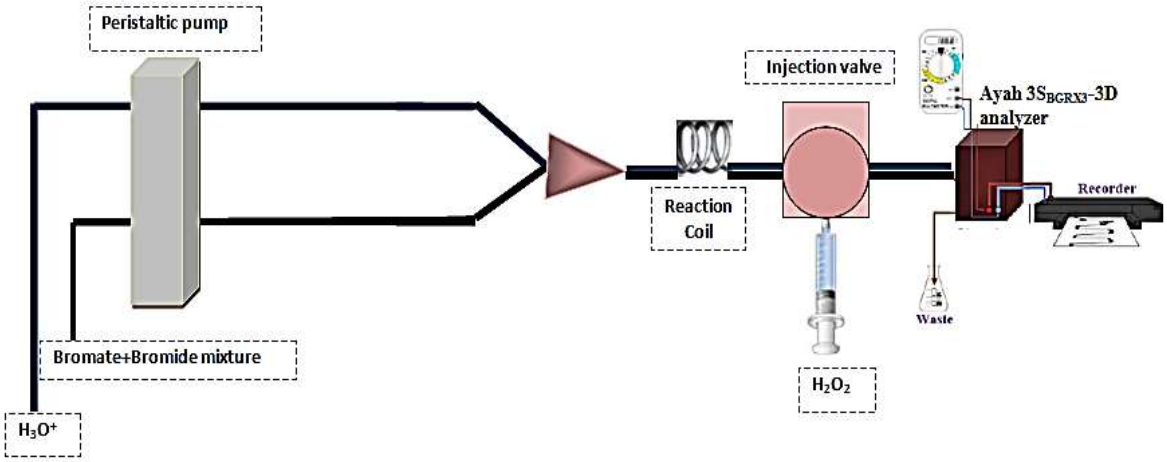


Figure 1: Flow System with Ayah3 S_{BGR} X3-3D-solar Cell for Determination of H_2O_2

$$5Br^- + 6H^+ + BrO_3^- \longrightarrow 3Br_2 + 3H_2O \quad (1)$$

$$Br_2 + H_2O_2 \longrightarrow Br^- + H_2O \quad (2)$$

RESULTS

Spectroscopic Study

The study was carried out to select the irradiation source from three sources represent the main additive color of the spectrum i.e Blue 470nm, Green 525nm and Red 635nm. The results obtained (fig.2) shows the most suitable light source was blue (470nm) used for the determination hydrogen peroxide.

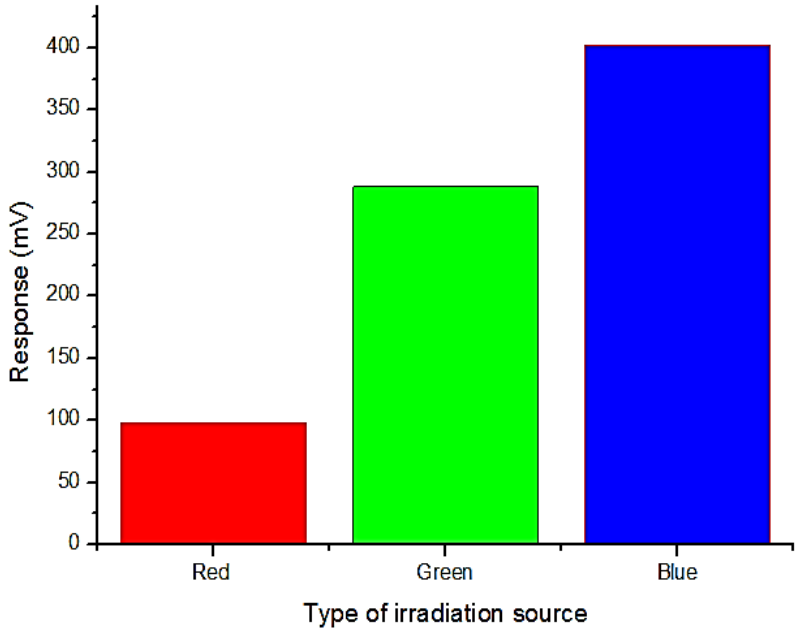
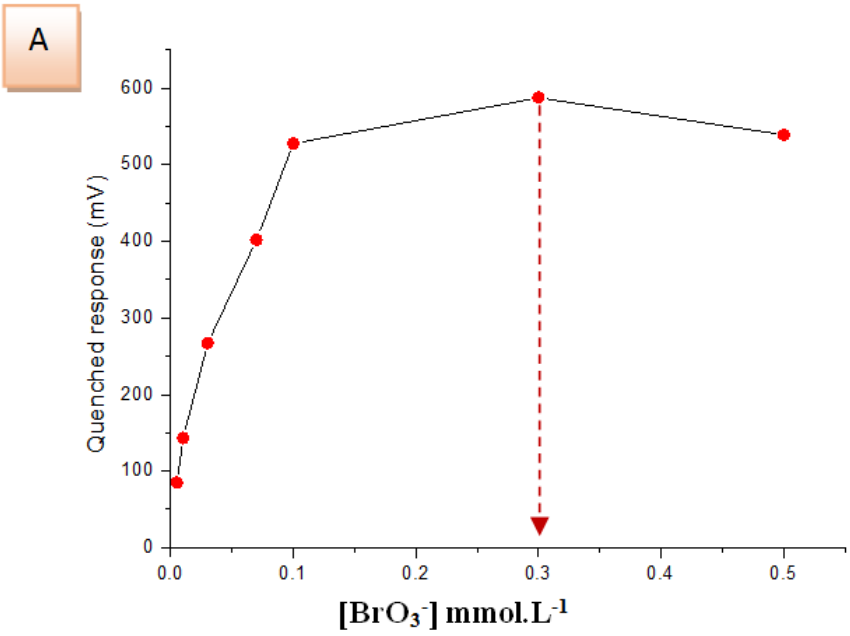


Figure 2: A Bar Representation for Different Light Emitting Diode Using 0.07mmol.L^{-1}BrO_3^--0.02mmol.L^{-1} Br^--0.04mmol.L^{-1}H_3O^+ (flow rate:1.3mL.min^{-1})

Effect of Bromate and Bromide Concentrations

Series of bromate ion concentration 0.005-0.5 mmol.L^{-1} at constant concentration of bromide ion (0.02 mmol.L^{-1}) with experimental chemical and physical conditions includes: [H_2O_2] : 7mmol.L^{-1} (85µL sample volume), [HNO_3]:0.04 mmol.L^{-1} and 1.3mL.min^{-1} flow -rate for line no.1 and line no.2 was used. Fig.3.A shows the variation of quenched absorbance response(mV) with the different bromate concentrations. A $KBrO_3$ concentration of 0.3 mmol.L^{-1} was chosen for optimum sensitivity, reproducibility and to avoid the higher concentrations of $KBrO_3$ because account the difficulty of dissolving of bromate ion >0.3mmol.L^{-1} with no increase in sensitivity.

After selected the optimum concentration of bromate ion a series of bromide ion solutions 0.01-0.4 mmol.L^{-1} were prepared. The results obtained indicates 0.08 mmol.L^{-1} KBr which chosen because the concentration of KBr up to 0.08 mmol.L^{-1} decrease of quenched absorbance which is attributable to the consumption that occurs in hydrogen peroxide with the increase of bromide ion concentration (Fig.3.B).



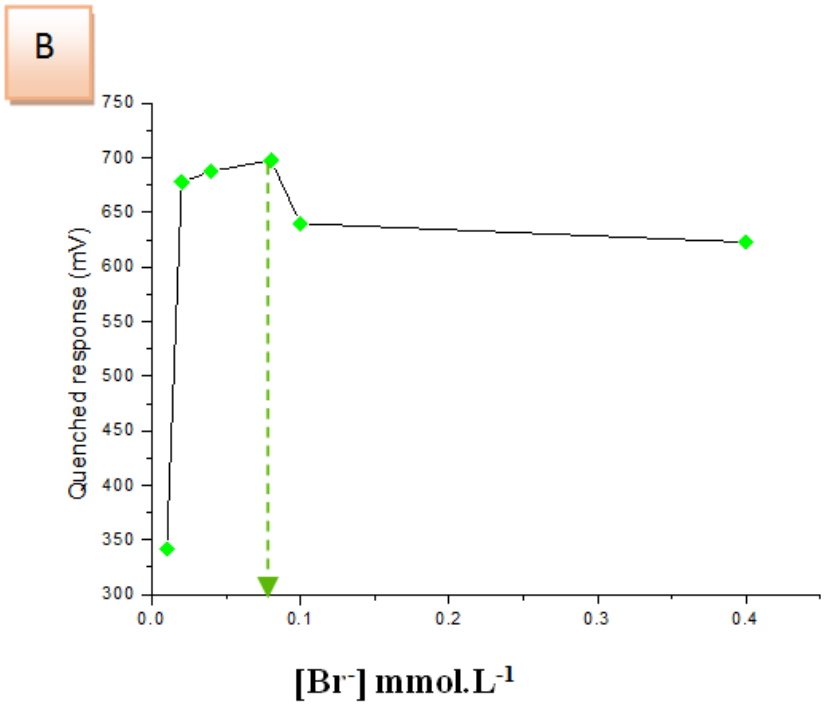


Figure 3: Relation between Output of Quenched Response with: A-Bromate Concentration B-Bromide Concentration

Type of Acid Medium

The optimum concentration of bromate and bromide ions with different acidic medium (HCl, HNO_3, H_2SO_4 and CH_3COOH at 0.04 mmol.L^{-1}) were used for the selection of better acidic solution to release bromine from the reaction between Br^- with H_3O^+ in the presence of BrO_3^- to determine of hydrogen peroxide. From the output of quenched response obtained (fig.4.A) it was found that nitric acid was given a highest and sensitive response this can be attributed to the degree of ionization of the acid used (the number of H_3O^+ ions produced by each acid).

After this step various nitric acid concentrations(0.03-0.4 mmol.L^{-1}) were prepared by appropriate dilution of the stock solution of HNO_3. From this experiment it can be concluded that the best concentration of nitric acid is 0.3mmol.L^{-1} (see fig.4.B)

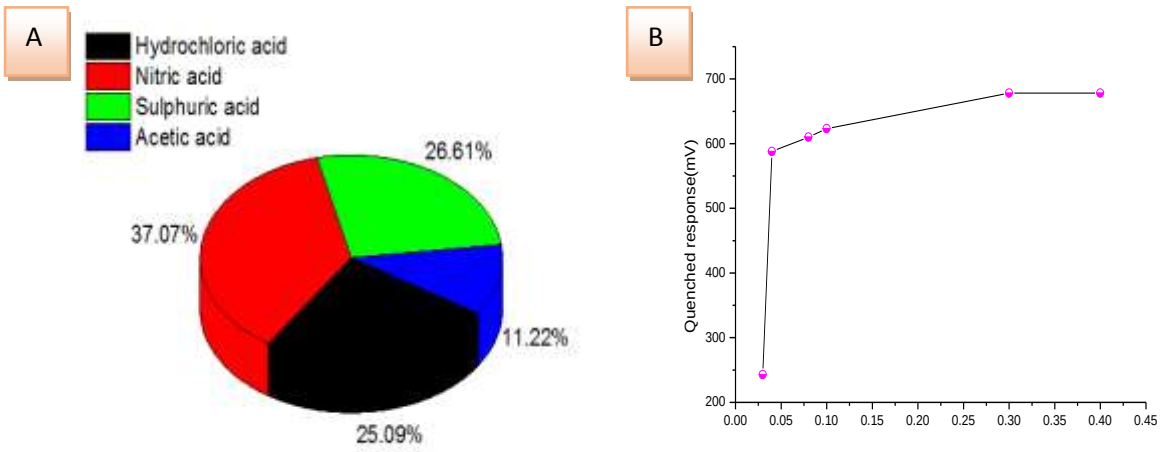


Figure 4: A-Pie Graph Represent the Contribution of each Acid B- Relation between Output of Quenched Response with Nitric Acid Concentration Flow Rate and Sample Loop

Variable flow-rate 1.0-2.3mL.min^{-1} were employed**.** The experimental parameters for maximum quenched absorbance response for the determination of hydrogen peroxide were used,BrO_3^- ; 0.3 mmol.L^{-1},Br^-;0.08 mmol.L^{-1},HNO_3;0.3 mmol.L^{-1};H_2O_2;7mmol.L^{-1}(85µL). A flow-rate of 1.6mL.min^{-1} (for line no.1 and line no.2) was found to give the regular response, better sensitivity, enough time and little dilution effect to determination of hydrogen peroxide(fig.5.A).

To establish the optimum sample volume, the volume of hydrogen peroxide sample was varied between of 25-200µL using open valve mode. Fig.5.B shows the decrease of output of quenched response with increase of the base under the peak (broadening the response) when increase of sample volume >85µL so 85µL was chosen as the best sample loop for the determination of hydrogen peroxide.

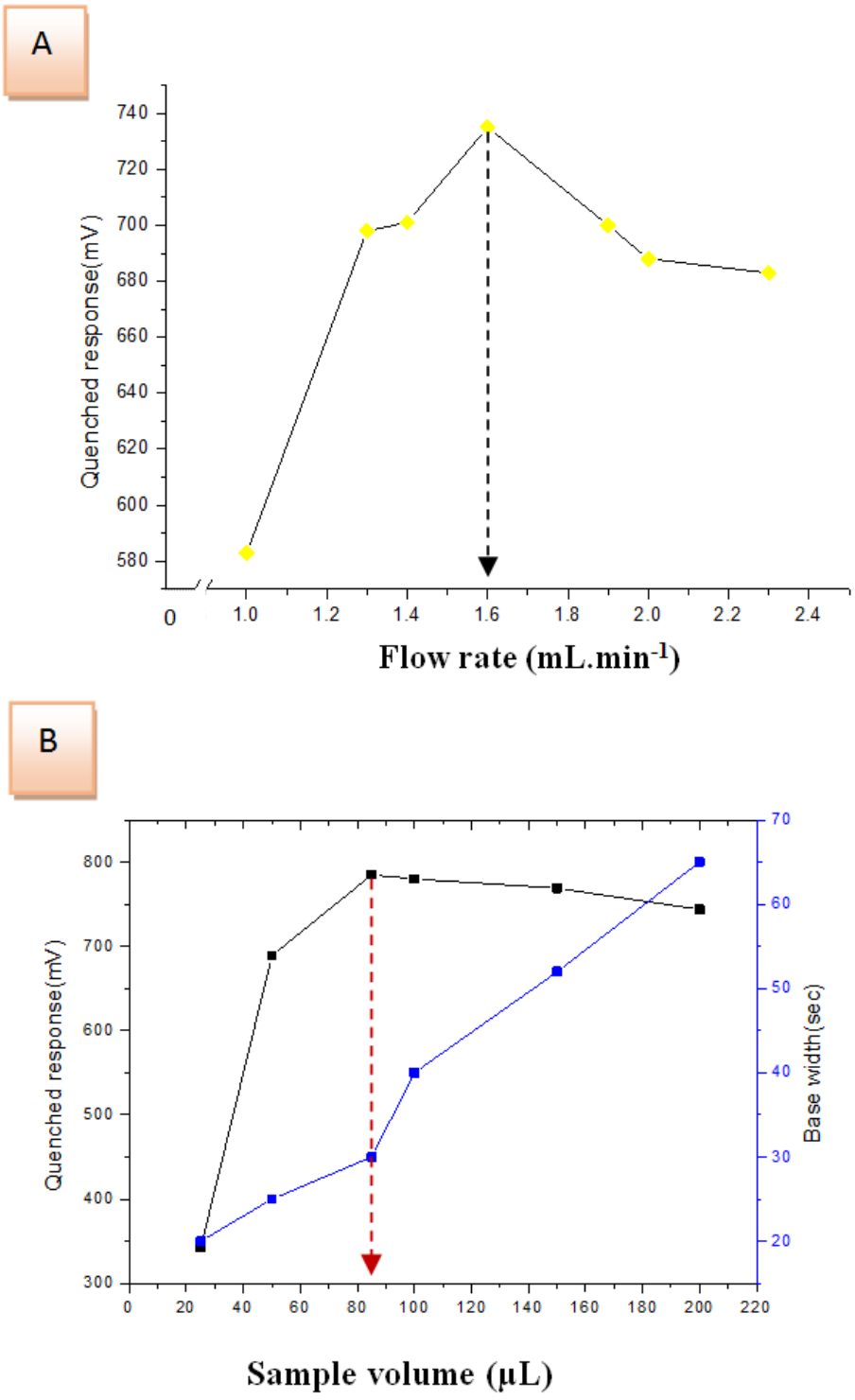


Figure 5: Quenched Absorbance Response Versus: A-Variation of Flow Rate B-Variation of Sample Volume

Delay Reaction Coil

Delay reaction coil 0-350cm which conducted after junction point directly to flow system were studied. 100cm give highest response and more homogeneity for the complete generation of bromine compared with other response. Therefore, a 100cm delay reaction coil was used in the first reaction to determination of hydrogen peroxide by quenched the continuous absorbance response.

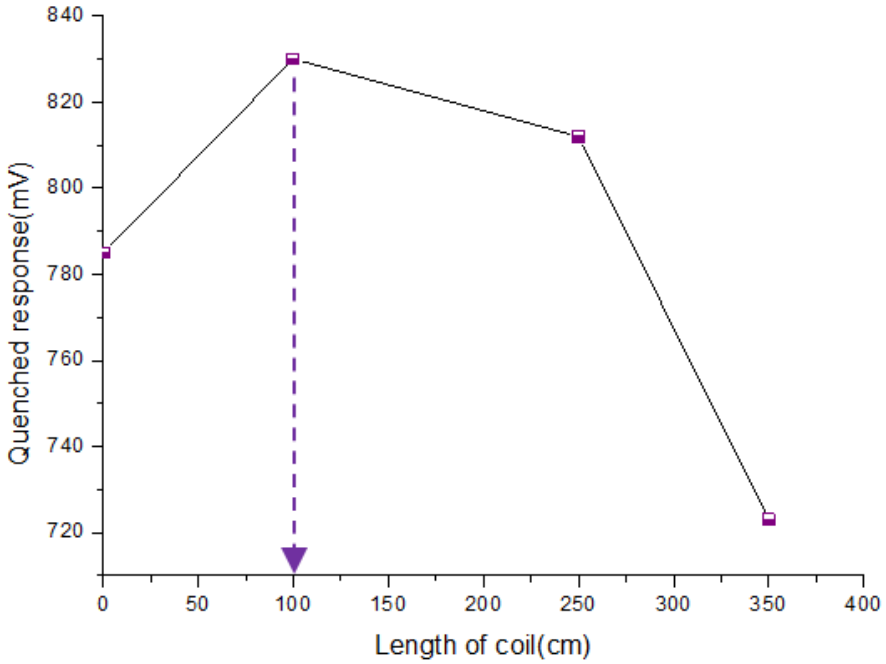


Figure 6: Effect of Delay Reaction Coil Versus Quenched Absorbance Response

Calibration Graph and L.O.D

Prepared a series of hydrogen peroxide solutions using the optimal conditions of the chemical and physical parameters mentioned in previous studies. The assay results were tabulated in table 1 which shows the equation of the straight line with r, r^2, R^2% at confidence interval 95%.

Successive dilution of 0.01mmol.L^{-1} hydrogen peroxide solution were carried out for calculation limit of detection theoretically and practically as tabulated in table 1. Table 2 shows the ANOVA[18] summary for the linear equation with treatment data of calibration curve for proposed method. It was realized from the results that F_{cal}(1346.34)>> F_{tab} (4.84) which in turn indicate that there is a significant difference for the variance due to regression (shows the ideality of the linear equation model in representing results) and the error leading in high increase in F value which give the exact correlation in expressing all the practically results using simple linear equation

Table1: Summary of calibration graph results and detection limit for the determination of H_2O_2

Range of H_2O_2 mmol.L^{-1} (n= 13)	**$\hat{Y}_{(mV)}$=a±s_at+b±s_bt H_2O_2 mmol.L^{-1} at confidence level 95%,n-2**	**r r^2 R^2%**	**t_{tab} at 95%,n-2**	**$t_{cal} = \frac{\lvert r\rvert\sqrt{n-2}}{\sqrt{1-r^2}}$**	**Detection limit(L.O.D) (ng/85µL)**	
					Practically based on the successive dilution of 0.01mmol.L^{-1}	**Theoretically (based on slope)**
0.01-15	**125.93±32.84+97.52±5.85 [H_2O_2]mmol.L^{-1}**	**0.9959 0.9919 99.19**	**2.201<<36.69**		**14.45**	**6.96**

$\hat{Y}$=estimate value , r = correlation coefficient
R^2% = Linearity percentage, r^2= coefficient of determination (C.O.D)

Table 2: ANOVA results for linear regression treatment

Source	Sum of Squares	Df	Mean Square	$F_{statistic}$ S_1^2/S_2^2	$F^{1}_{11\ tab}$ at 95%
Regression	**2563002.3**	**υ_1=1**	**S_1^2=2563002.3 S_2^2=1903.679**	**1346.34**	**4.84**
Error	**20940.469**	**υ_2=11**			
Total	**2583942.8**	**12**			

Application

The spectrophotometer-flow injection analysis method was applied to the analysis of hydrogen peroxide in three pharmaceutical preparations. The standard addition method has been used to rely on the developed method and classical method(Turbidity $_{(0\text{-}180)}$). The analytical results of both methods were treated statistically by using paired t-test at 95% (confidence limit) which shown that there is no significant difference between two methods.

Table 3: Results for standard addition procedures for the determination of [H_2O_2]

No. of sample *	[H_2O_2] Percentage(%)	After standardization [H_2O_2] percentage(%)	Volume draw to prepare 5mmol.L^{-1} (100mL)	$\hat{Y}_{(mV)}=a\pm s_a t+b\pm s_b t$ H_2O_2 mmol.L^{-1}	Practical concentration mmol.L^{-1} (at origin sample)	Practical percentage% Recovery %
1	5.88mol.L^{-1} (20%)	2.58 15%	0.19	66.6±16.55+646± 67.55	2.71	9.21% 105.06%
2	7.35mol.L^{-1} (25%)	3.23 20%	0.15	43.0±4.49+390±1 8.37	3.66	12.44% 113.31%
3	2.06mol.L^{-1} (7%)	1.22 5%	0.41	54.2±13.71+492± 55.97	1.341	4.56% 109.95%

***Samples:1-Al-Amire(Syria), 2-Baghdad (Iraq) and 3-Al-Areje (Iraq)**

Table 4: Summary of results for paired t-test for determination of hydrogen peroxide in three samples

No. of sample	Practical percentage(%)		Xd	$\bar{x}_d$ (σ_{n-1})	$t_{cal}=\bar{x}_d\sqrt{n}/\sigma_{n-1}$ at 95 %	t_{tab}
	Spectrophotometer	Turbidity				
1	9.21	8.77	0.44	0.77 (0.598)	2.23<<4.303	
2	12.44	10.98	1.46			
3	4.56	4.15	0.41			

Xd: Difference between two method , $\bar{x}_d$: difference mean , σ_{n-1}:Difference standard deviation

CONCLUSION

The developed method has been applied successfully to the determination of hydrogen peroxide with a detection limit 14.45ng/sample.The continuous absorbance measured by Ayah3S_{BGR} x3-3D-solar cell when the combination of BrO_3^--Br^--H_3O^+ because the release of bromine; hence the injected H_2O_2 quenched the response obtained because converted the solution from red-brown to colorless solution as a result of the formation of bromide ion species. The method is simple, fast and high reproducible. Future work on the determination of acidity is in progress, using the same principle, and also on the determination of the bromate and bromide ions.

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REFERENCES

[1] W.C., Satterfield, C.N., & R.L. (1955). *Wentworth, in 'Hydrogen Peroxide',* Rheinhold Publ. Co., New York.

[2] Riley. (1963). *Handbook of preparative inorganic chemistry*, 1, 2nd Ed., N.Y.: Academic Press, New York.

[3] Grant, W.M. (1974). *Toxicology of the Eye; Drugs, chemicals, plants, venom,* 2nd Ed., C. C. Thomas, Springfield, Illinois.

[4] Saeyer. (1991). *Oxygen Chemistry,* Oxford university press, Inc., New York.

[5] Daiyao, W., Shiyi, Q., Mengyun, W., Siwen, P., Hongfang, M., & Jing, Z. (2019). Spectrophotometric determination of hydrogen peroxide in water by oxidative decolorization of azo dyes using Fenton system. *Spectrochimica Acta part A: Molecular and Biomolecular Spectroscopy, 221*(5): 117138.

[6] Huahua, C., Xin, L., Jing, Z., Junyang, X., Baoling, Y., Fei, L., & Qingfeng, C. (2018). Multi-wavelength spectrophotometric determination of hydrogen peroxide in water with peroxidase-catalyzed oxidation of ABTS. *Chemosphere, 193*: 833-839.

[7] Bahar, G., Aysem, Ù., Selen, D., Erol, E., & Resat, A. (2019). Titanium dioxide nanoparticles-based colorimetric sensors for determination of hydrogen peroxide and triacetone triperoxide (TATP). *Talanta, 202*(1): 402-410.

[8] Laura, D., Andrea, T., Damiano, M., & Manuela, A. (2018). Assessment of a colorimetric method for the measurement of low concentrations of peracetic acid and hydrogen peroxide in water. *Talanta, 183*(1): 209-215.

[9] Meng, M., Xin, L., Hui, L., Zi-Zhen, G., Hui-Hong, H., Yun, L., Hua-Ping, P., Wei, C., Yin-Hua, L., Ai-Lin, L., & Xing-Hua, X. (2019). A colorimetric assay for sensitive detection of hydrogen peroxide and glucose in microfluidic paper-based analytical devices integrated with starch-iodide-gelatin system. *Talanta, 200*(1): 511-517.

[10] Anastasia, S., Anna, D., Sergey, V., & Konstantin, A. (2019). Method for determination of hydrogen peroxide in adulterated milk using high performance liquid chromatography. *Food Chemistry, 283*(15): 431-436.

[11] Jeyaraman, S., & Gymama, S. (2019). Towards a dual in-line electrochemical biosensor for the determination of glucose and hydrogen peroxide. *Bioelectrochemistry, 128:* 56-65.

[12] Nagam, S., & Ahmed, F. (2015). Determination of hydrogen peroxide in some local pharmaceutical disinfectants by continuous flow injection analysis via turbidimetric (T180o) and scattered light effect at two opposite position (2N90o) using Ayah 4SW-3D-T180o -2N90o-Solar-CFI analyser. *Iraqi Journal of Science, 56*(1C): 577-592.

[13] Issam, M., & Nagam, S. (2000). New mode of on-line automation analysis: chemiluminescence determination of hydrogen peroxide using continuous flow and chemiluminescence detection via generation of luminol chemiluminescence. *Iraqi Journal of Science, 41A* (3): 29-47.

[14] Issam, M (2015). New mode of on-line automation: chemilumino-fluoro metric method for the determination of hydrogen peroxide by fluorescence energy transfer using poly acrylic acid gel beads. *Iraqi Journal of Science, 56*(2B): 1289-1302.

[15] Issam, M., & Nagam, S. (2001). New mode of on-line automation determination of hydrogen peroxide by total luminescence utilizing the chemiluminescence energy released from luminol oxidation as internal source for irradiation of Rhodamine-B molecule. *Iraqi Journal of Science*, *42A*(2): 23-57.

[16] Nagam, S. (2019). A nobel chemiluminescence and fluorescence energy transfer three inlets cell for the determination of hydrogen peroxide via continuous flow injection analysis. *Journal of Global Pharma Technology, 11*(5): 484-494.

[17] Issam, M.A.S., & Nagam, S.T. (2012). Ayah 3SX3-3D- Solar Analyser, *Patent*, G01N29/00.

[18] Miller, J.C., & Miller, J.N. (2010). *Statistics and chemometrics for analytical chemistry,* 6th Ed., Pearson education limited, England.