ISSN: 0067-2904
GIF: 0.851

Determination of hydrogen peroxide in some local pharmaceutical disinfectants by continuous flow injection analysis via turbidimetric (T_{180}^{o}) and scattered light effect at two opposite positions ($2N_{90}^{o}$) using Ayah $4S_W$-3D-T_{180}^{o} -$2N_{90}^{o}$ -Solar - CFI Analyser

Nagam S. Turkey Al-Awadie[1*], Ahmed F. Khudhair[2]
[1]Chemistry department-College of Science-University of Baghdad- Baghdad-Iraq
[2]Chemistry department-College of Science-University of kerbala –kerbala -Iraq

Abstract

An indirectly method is used to determine hydrogen peroxide. The method based on oxidation of chromium (III) ion by hydrogen peroxide in basic medium to form chromate ion which react with barium (II) ion to produce a yellow precipitate ($BaCrO_4$). Under the optimum established conditions, the linear range of 0.50-25.00 mmol L^{-1} along with correlation coefficient (r) of 0.9992, Limit of detection (LOD) 0.68 µg / 100 µL, precision expressed as relative standard deviation for six replication measurements at 5.0 mmol.L^{-1} H_2O_2 of less than 2% were obtained for hydrogen peroxide. The developed method was successfully applied for the estimation of H_2O_2 in three pharmaceuticals preparation of different companies using continuous flow injection or stop-flow injection technique.

Key word: Hydrogen peroxide, Turbidity & Nephelometry, Flow injection analysis.

تقدير بيروكسيد الهيدروجين في بعض المطهرات الصيدلانية باستخدام تحليل الحقن الجرياني المستمر عن طريق قياس التعكريه (T_{180}°) وتأثير استطاره الضوء عند اتجاهين متعاكسين ($2N_{90}^{\circ}$) باستخدام المحلل Ayah $4S_W$–3D–T_{180}^{o} –$2N_{90}^{o}$ –Solar – CFI

نغم شاكر تركي العوادي[1*]، أحمد فاضل خضير[2]
[1] قسم الكيمياء ، كلية العلوم ، جامعة بغداد ، بغداد ، العراق
[2] قسم الكيمياء، كلية العلوم، جامعة كربلاء، كربلاء، العراق

الخلاصة

تم التقدير غير المباشر لبيروكسيد الهيدروجين من خلال تكوين راسب أصفر من كرومات الباريوم الناتج من اكسدة آيون الكروم (III) بأستعمال بيروكسيد الهيدروجين في الوسط القاعدي لتكون آيون الكرومات الذي يتفاعل مع آيون الباريوم (II) اذ كان مدى الخطية لمحلول بيروكسيد الهيدروجين بين (25.00−0.50) مللي مول.لتر$^{-1}$ مع معامل الارتباط (r) 0.9992 ، تم الحصول على حد كشف (L.O.D) 0.68 مايكروغرام\ 100 مايكرولتر و الانحراف القياسي النسبي المئوي لتركيز 5.0 مللي مول.لتر$^{-1}$ أقل من 2% (n = 6). طبقت الطريقة بنجاح لتقدير بيروكسيد الهيدروجين في ثلاث مستحضرات صيدلانيه(شركات مختلفه) باستخدام جريان مستمر او جريان توقف.

---

*Email: nagamturkey2013@gmail.com

Introduction

Hydrogen peroxide was discovered in 1818 by French chemist Louis-Jacques Thenard finally succeeded in preparing the pure hydrogen peroxide, which named it “oxygenated water” and determined its density [1, 2]. For a long time it was believed that pure hydrogen peroxide is unstable, due to incapability to separate the hydrogen peroxide from the water. This was because traces of solids and heavy metal ions led to a catalytic decomposition or explosions of the hydrogen peroxide, 100% pure hydrogen peroxide was first obtained through vacuum distillation by Richard Wolffenstein in 1894[3].

Hydrogen peroxide is a clear, colorless liquid with a characteristic odour that it always exists in aqueous solution at different concentrations [4]. At high concentrations, hydrogen peroxide causes irritation to the eyes and skin and affects human health [5, 6]. Hydrogen peroxide plays a significant role in the chemical and pharmaceutical industries [7]. Further, the detection of hydrogen peroxide is an important task in many biological, medical and clinical studies [8]. Hydrogen peroxide is used as oxidant, disinfectant and bleaching agent in various industrial and household applications [9, 10]. Literature survey shows that various analytical methods have been reported for the determination of hydrogen peroxide due to the fact that hydrogen peroxide plays significant role in many fields including food, pharmaceutical, chemical, biochemical industries, clinical control and environmental protection [11, 12].

This paper describes a flow injection (FI) turbidimetric and nephelometric method for determination of hydrogen peroxide in some local pharmaceutical disinfectants. The method is based on oxidation of Cr (III) ion by hydrogen peroxide in basic medium to form chromate ion which react with Ba (II) ion to produce a yellow precipitate ($BaCrO_4$). The precipitate is measured via the attenuation of incident light, also scattering of the incident light in $+90^o$ and -90^o angle were measured.

Experimental

Chemicals

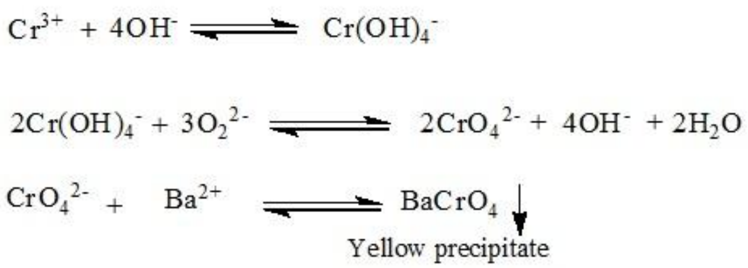
All chemicals were used of analytical-reagent grade while distilled water was used to prepare the solutions. A stock solution hydrogen peroxide (35% H_2O_2, 34.01, Solvay, 2mol L^{-1}) was prepared by diluting 97.46 mL in 500 mL of distilled water (standardized with standard $KMnO_4$ solution). Sodium hydroxide solution (NaOH, 40, Fluka, 1 mol L^{-1}) was prepared by dissolving 40g in 1L (standardized with HCl solution). A standard chromic chloride solution ($CrCl_3.6H_2O$, 266.46, BDH, 0.1 mol L^{-1}) was prepared by dissolving 26.6460 g in 1L and a standard barium chloride ($BaCl_2.2H_2O$, 244.28, BDH, 0.5 mol L^{-1}) was prepared by dissolving 30.5350 g in 250 mL.

Apparatus

Peristaltic pump – 4 channels (Switzerland) an Ismatic type ISM796. A rotary 6- port injection valve (Teflon) ,(IDEX corporation, USA).The response was measured by a homemade Ayah $4S_W$-$3D$-T_{180} - $2N_{90}$ - Solar - CFI Analyser [13] ,which uses four white snow LED for irradiation of the flow cell at 2mm path length. Three solar cells were used as a detector for collecting signals via travelling of sample for 40 mm length. The readout of the system composed of x-t potentiometric recorder (KOMPENSO GRAPH C-1032) SIEMENS (Germany) or digital AVO-meter (auto range) (0.00-2000 mV) (China).

Methodology

The two lines manifold system Figure -1was used, the first line supplied by using Cr(III) (19 mmol L^{-1}) as the carrier (2.4 mL min^{-1}) while the injected sample consist of mixture from hydrogen peroxide (0.025 mol L^{-1}) with hydroxide ion solution (0.1 mol L^{-1}), the injected sample volume of H_2O_2 was used 200 µL that passes through reaction coil length (0, 10, 30 , 40, 50 and 60 cm) completion of reaction i.e. conversion of Cr (III) (green) to Cr (VI) (yellow) as chromate; while in turn mixes with Ba (II) ion(7.3 mmol L^{-1}) (2.4 mL min^{-1}) at the junction point to form the yellow precipitate as shown in the written chemical equations (see below)[14]. Coil length of 50 cm (1570 µL) was most favorable choice for the completion of the conversion of Cr (III) to Cr (VI) as chromate ion CrO_4^{2-}.



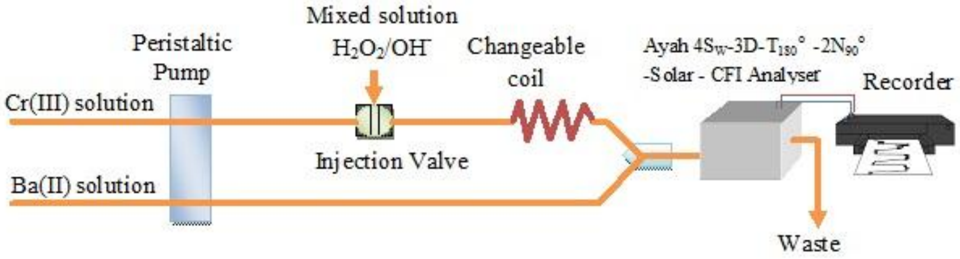


Figure 1- Flowgram for hydrogen peroxide determination in two lines by using mixed solution (NaOH /H_2O_2) as the injected sample.

Variable Optimization

The chemical parameters (mainly concentration and pH of the reaction medium) as well as physical parameters (flow rate, sample volume, time purge, and intensity of incident light p°) were studied.

Chemical Variables

Chromium (III) Concentration

A series of Cr(III) solutions (0.5-100.0 mmol L^{-1}) as carrier stream (2.4 mL min^{-1}) were prepared. 0.025 mol L^{-1} of H_2O_2 was used, 200 µL sample volume in basic medium (0.1mol L^{-1} of NaOH) and second line 7.3 mmol L^{-1} of Ba (II) at 2.3 mL min^{-1} flow rate and the intensity of incident light of the total four LEDs is 1100 mV. The total results obtained were tabulated in Table-1 represented via four x-t potentiometric recorder (attenuation of incident light, scattering of light in two way and outcome of scattering light (electrical outcome). It can be seen that an increase in concentration of Cr(III) ion causes an attenuation of incident light due to accumulation and compactness of the precipitated particles causing obstruction of the incident light which led to increase of attenuation of the incident light while there is an increase at N_L due to the reflections of the light from particles surfaces , same behavior is observed in N_R as shown in Figure -2A, up to 19mmol L^{-1} Cr(III) concentration was chosen as the optimum concentration that used for further experiments. Figure -2B shows the response profile at optimum chromium (III) concentration. The concentration of Cr (III) larger than 19 mmol L^{-1} cause to decrease of attenuation of incident light and reflection in two ways (±90°) and algebraic sum of them. It is probable that it could be due to the restriction of the passage of incident light due to the accumulation of precipitate particles in front of the detector.

Table 1-Effect of chromium (III) concentration on the measurement of attenuation of incident light (turbidity) as well as reflection of light at two opposite positions and algebraic sum of them.

[Cr(III)] mmol L^{-1}	Type of measurement $\bar{y}_i \pm t_{0.05/2}\, \sigma_{n-1}/\sqrt{n}$ (n=3) (mV)			
	Attenuation of incident light $T_{(0-180^o)}$	Scattering of light (L) N_L (+90°)	Scattering of light (R) N_R (-90°)	Algebraic sum of the scattering of light $N_{(L+R)}$ (±90°)
0.5	20±1.250	10±0.000	5±0.000	25±1.358
10.0	80±1.056	18±0.000	16±0.739	36±0.547
19.0	216±2.783	120±0.574	20±0.000	68±2.789
50.0	152±1.784	92±0.666	16±2.082	56±3.143
70.0	128±0.987	60±1.292	12±1.034	52±0.695
100.0	88±0.507	30±0.000	10±1.533	20±0.000

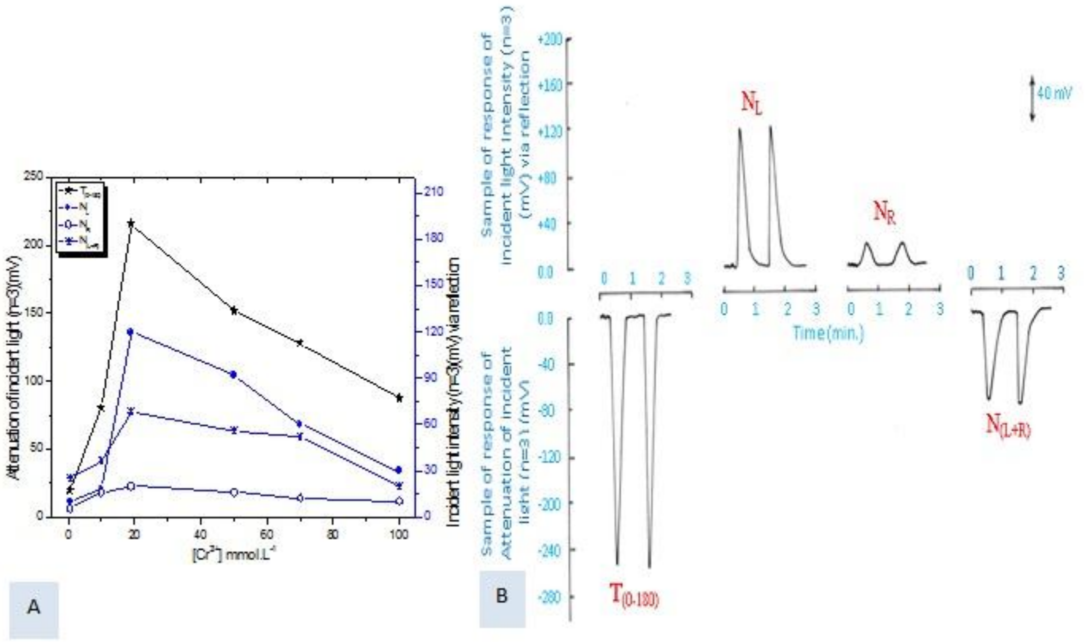


Figure 2-Effect of chromium (III) concentration on; (A) Attenuation of incident light, scattering of light at two opposite directions and the algebraic sum of them, (B) Response profiles at optimum chromium (III) concentration(19mmol L^{-1}).

Barium (II) Concentration

Effect of Ba (II) concentration was studied using 19 mmol L^{-1} of Cr (III); while maintaining other variables as in previous experiments are constant. Variable concentration (0.1- 15.0 mmol L^{-1}) of Ba (II) ion was used. The results tabulated in Table-2 which shows that an increase on the attenuation of incident light, scattering of light in two way and outcome of scattering light (electrical outcome) (±90^o) with increased Ba(II) concentration. A10 mmol L^{-1} was selected as the optimum concentration that can give a better reproducible outcome. Figure-3A shows the effect of variation of Ba(II) concentration on attenuation of light, scattering light (±90^o) and the algebraic sum of them, Figure-3B shows the response profile at optimum Ba (II) concentration.

Table 2-Effect of Ba(II) concentration on the measurement of attenuation of incident light as well as reflection of light at two opposite positions and algebraic sum of them. 200µL, 0.025 mol L^{-1} H_2O_2.

[Ba(II)] mmol L^{-1}	Type of measurement $\bar{y}_i \pm t_{0.05/2,\,n-1}\ \sigma_{n-1}/\sqrt{n}$ (n=3) (mV)			
	Attenuation of incident light $T_{(0-180^o)}$	Scattering of light (L) N_L (+90^o)	Scattering of light (R) N_R (-90^o)	Algebraic sum of the scattering of light $N_{(L+R)}$ (±90^o)
0.1	220±2.130	20±0.437	6±0.000	12±0.566
0.5	230±1.776	22±0.000	8±0.974	14±0.985
1.0	250±3.052	26±0.567	10±2.241	16±0.000
5.0	300±2.328	36±1.579	12±1.152	20±0.933
10.0	316±1.652	40±1.383	14±2.781	24±1.391
15.0	308±1.489	36±1.022	12±1.035	20±1.562

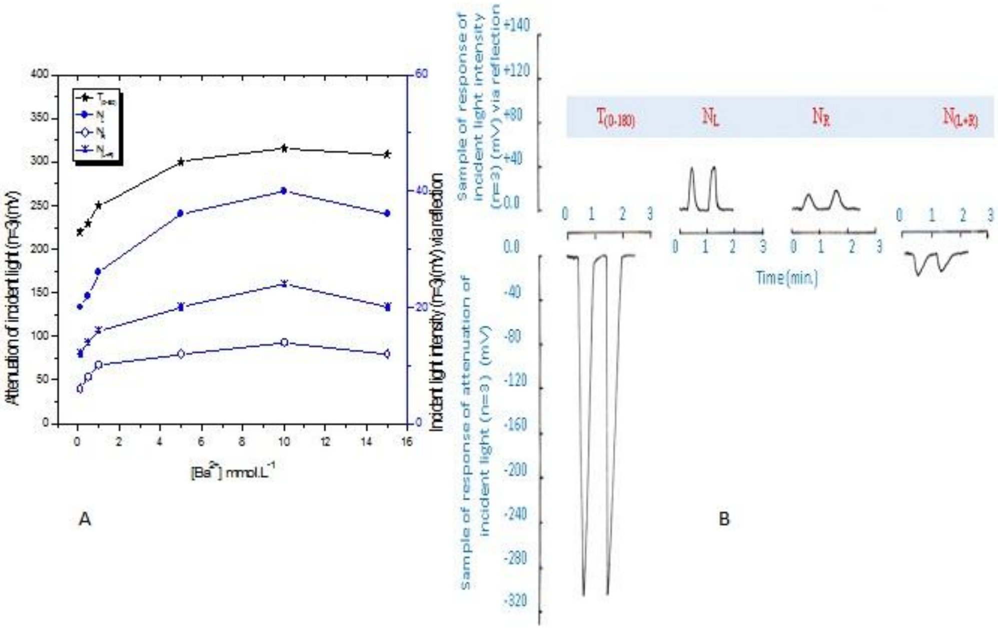


Figure 3-Effect of Ba (II) ion concentration on; (A) Attenuation of incident light, scattering of light at two opposite directions and the algebraic sum of them. (B) Response profiles at optimum Ba (II) concentration.

Sodium Hydroxide Concentration

The effect of the sodium hydroxide concentration on the sensitivity in general was studied. A series of solutions (0.05- 0.5 mol L^{-1}) were prepared while keeping up other variables as in previous experiment are constant. Table-3 summarized the total results obtained which shows the effect of the increase of sodium hydroxide concentration on the sensitivity of response (up to 0.15mol L^{-1}) to generate the $Cr(OH)_4^-$ complex ion that oxidize to CrO_4^{2-}. At more than 0.15mol L^{-1} NaOH an increase $BaCrO_4$ precipitate causing obstruction of incident light leading to decrease of scattered light N_L, N_R and N_{L+R} and cause to irregular response profile, i.e. 0.15 mol L^{-1} NaOH concentration was regarded as the optimum concentration and used for further work.

Table 3-Effect of NaOH concentration on the measurement of attenuation of incident light as well as reflection of light at two opposite positions also algebraic sum of them, 200 µL, 0.025 mol $L^{-1}H_2O_2$.

[NaOH] mol L^{-1}	Type of measurement $\bar{y}_i \pm t_{0.05/2,\, n-1}\ \sigma_{n-1}/\sqrt{n}$ (n=3) (mV)			
	Attenuation of incident light $T_{(0-180^o)}$	Scattering of light (L) N_L (+90°)	Scattering of light (R) N_R (-90°)	Algebraic sum of the scattering of light $N_{(L+R)}$ (±90°)
0.05	60±2.532	10±0.000	4±0.000	8±1.321
0.10	300±4.451	22±0.562	8±0.000	14±0.546
0.15	332±3.259	30±0.000	15±1.221	20±0.000
0.25	320±2.561	26±0.883	16±0.629	20±0.883
0.50	360±1.980	24±1.406	18±0.564	23±1.117

Physical Variables

Flow Rate

Using optimum chemical parameters and experimental physical parameters achieved in previous sections. A variable flow rate from 0.78 to 3.70 mL min^{-1} was studied. The results were tabulated in Table -4. It can be noticed that at low flow rate there is an increase in dispersion and dilution due to the increase of area of precipitate section in flow cell that causes a long time merging of precipitated section in front of detectors and an increase the attenuation and scattering of incident light and more dispersion on profile response. While an increase of flow rate results in a decrease of sample section , in addition to compact of merged layers due to rashness of successive travelled sample segment zones producing an effect similar to Doppler effect [15] causing an increased effect of attenuation resulting output of energy transducer .

Table 4-Effect of flow rate (two lines streams) on the measurement of attenuation of incident light as well as reflection of light at two opposite positions and algebraic sum of them.

Speed of peristaltic pump (indication approximate)	Flow rate (mL/min)		t (sec)	Type of measurement $\bar{y}_i \pm t_{0.05/2,\, n-1}\ \sigma_{n-1}/\sqrt{n}$ (n=3) (mV)			
	Ba(II) stream	carrier stream		Attenuation of incident light $T_{(0\text{-}180^o)}$	Scattering of light (L) N_L (+90°)	Scattering of light (R) N_R (-90°)	Algebraic sum of the scattering of light $N_{(L+R)}$ (±90°)
10	0.78	0.78	108	384±3.116	26±2.544	16±2.182	22±0.522
20	1.48	1.55	48	318±2.931	22±0.977	12±3.463	20±0.000
30	2.30	2.40	36	296±2.673	21±1.421	12±1.957	18±0.439
40	3.00	3.15	24	298±1.137	20±0.000	11±1.081	17±0.951
50	3.63	3.70	18	245±1.520	16±0.592	8±1.310	14±1.328

t(sec.) : Arrival time of sample segment from injected valve reaching to the measuring flow cell depend on $T_{0\text{-}180^o}$.

Figure-4 shows 2.40 & 2.30 mL min^{-1} for that carrier stream and Ba(II) line respectively is the optimum flow rate which is chosen on the basis of better sensitivity and repeatability. At high flow rate a decrease in sensitivity responses of attenuation of incident light as well as reflection of light at two opposite positions due to the incomplete oxidation process for the formation of CrO_4^{2-}, which might cause to a decrease of precipitated particle formation.

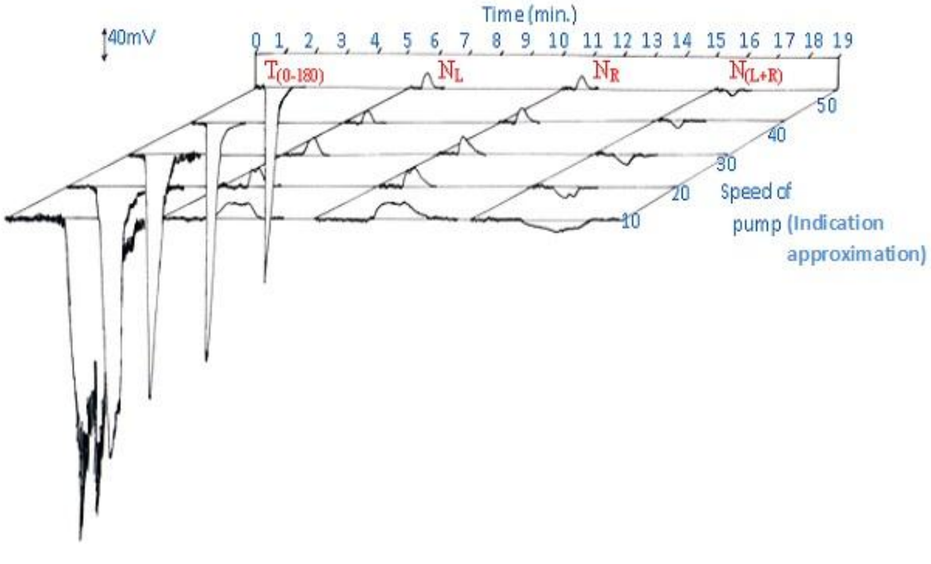


Figure 4- Effect of flow rate on attenuation of incident light, scattering of light at two opposite directions and the algebraic sum of them.

Sample Volume

The injected volume of sample was varied from 40 to 200 µL by changing the length of the sample loop in the injection valve, while the other chemical and physical parameters were remained fixed. An increase in the injection volume led to a significant increase in sensitivity and more perceptible than low volumes as shown in Table -5 and Figure-5A, B which shows that the optimum sample volume be of 100 µL giving a regular responses of the attenuated light and scattering of light (±90). Using sample volume larger than 100 µL gave a good response with wider Δt_B of response. In addition, continuation of the passage of carrier stream through the injection valve will cause an increase in the dispersion for the precipitated particles that cause a long duration of the precipitate in the flow cell and an increased exposure time in front of the detector.

Table 5- Effect of sample volume on the measurement of attenuation of incident light as well as reflection of light at two opposite positions and the algebraic sum of them.

length of sample loop(cm), i.d(mm)	Sample volume (µL)	t(sec.)	Type of measurement $\bar{y}_i \pm t_{0.05/2,\, n-1}\ \sigma_{n-1}/\sqrt{n}$ (n=3) (mV) / Δt_B (sec.)			
			Attenuation of incident light $T_{(0-180^o)}$	Scattering of light (L) N_L (+90°)	Scattering of light (R) N_R (-90°)	Algebraic sum of the scattering of light $N_{(L+R)}$ (±90°)
20.4 (0.5mm)	40	24	60± 0.000 18	8±0.632 12	4±0.000 6	8±0.000 18
41.0 (0.5mm)	80	30	276±1.006 21	36±3.425 30	8±0.000 15	20±2.673 20
51.0 (0.5mm)	100	39	332±1.541 30	48±2.192 33	10±0.460 18	24±1.661 30
61.0 (0.5mm)	120	42	300±2.315 36	40±2.839 36	8±0.000 30	20±1.009 33
45.3 (0.75 mm)	200	36	270±0.992 42	20±1.792 42	10±0.000 40	12±1.658 42

t(sec.): Arrival time of sample segment from injected valve reaching to the measuring flow cell depend on T_{0-180^o}.

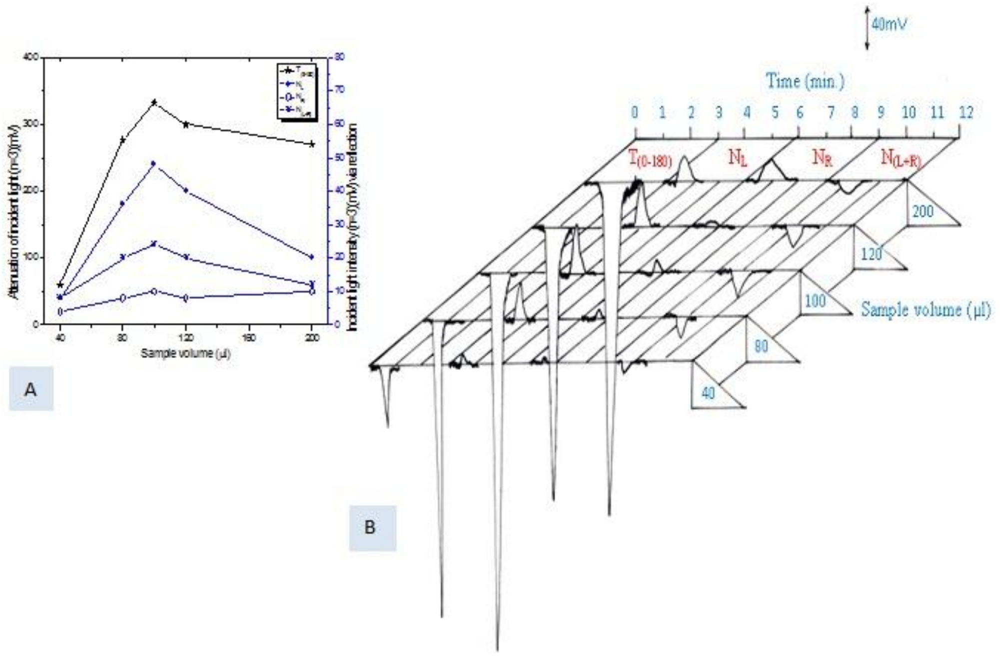


Figure 5-Effect of sample volume on; (A) Attenuation of incident light, scattering of light at two opposite directions and the algebraic sum of them, (B) Response profiles.

Purge Time

Allowed permissible time 10, 15, 20, 30, 40, 50 and 60 seconds were used for this study. The optimum physical and chemical parameters achieved in previous sections were kept constant at their values. All results were summarized in Table -6. The results show that 15 sec is the best purge time because it gave the highest response for the attenuation of incident light as well as the scattering of incident light at ±90° (N_L and N_R) and the algebraic sum of them as shown in Figure- 6A,B.

Table 6- Effect of purge time on the measurement of attenuation of incident light as well as reflection of light at two opposite positions also algebraic sum of them.

Purge Time (sec.)	Type of measurement $\bar{y}_i \pm t_{0.05/2,\, n-1}\ \sigma_{n-1}/\sqrt{n}$ (n=3) (mV)			
	Attenuation of incident light $T_{(0\text{-}180^o)}$	Scattering of light (L)N_L (+90^o)	Scattering of light (R)N_R (-90^o)	Algebraic sum of the scattering of light $N_{(L+R)}$ (±90^o)
10	324 ±1.102	45±1.091	6±0.000	24±1.258
15	332±0.000	48±0.868	8±0.000	32±1.797
20	300±0.561	49±0.763	8±0.000	28±0.000
30	260±2.653	57±1.003	9±0.537	32±1.786
40	256±2.002	68±1.372	12±0.968	40±2.276
50	252±1.373	72±0.000	13±0.000	48±2.016
60	248±1.863	76±0.995	14±0.000	48±1.902

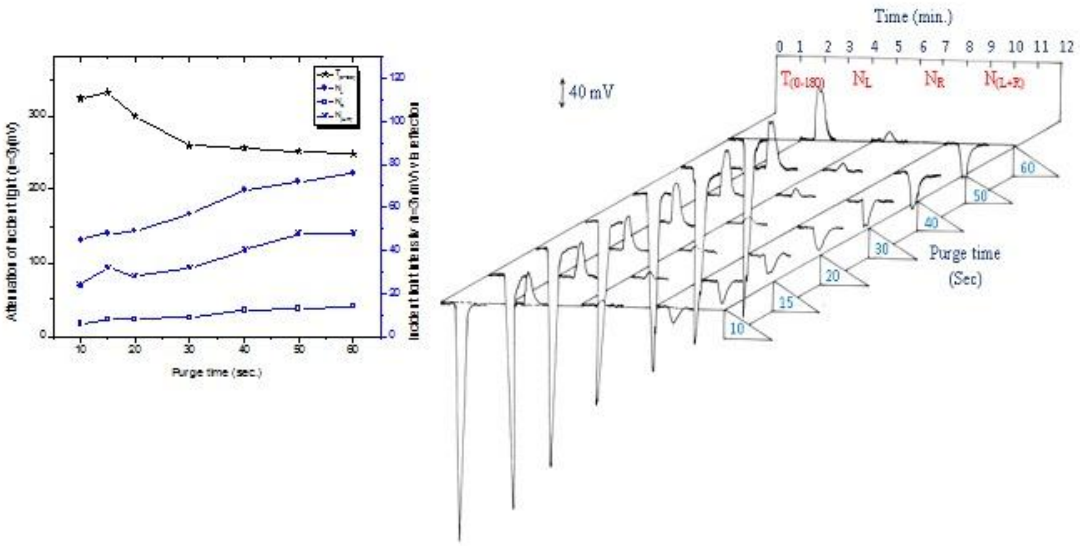


Figure 6- Effect of purge time on; (A) Attenuation of incident light, scattering of light at two opposite directions and the algebraic sum of them, (B) Response profiles.

Incident Light Intensity

Intensity of light source was studied while maintaining other chemical and physical parameters fixed. Variable intensity of light source was used in the range of 0.49– 1.95 Volt by variation of light intensity channel in Ayah $4S_W$-3D-T_{180^o} -$2N_{90^o}$ -Solar - CFI Analyser operation where read by AVO-meter. The results tabulated in Table -7 shows that an increase on the attenuation of incident light, scattering of light in two ways and outcome of scattering light (±90^o) with increased intensity of source light. The intensity of 1.74 Volt was selected as the voltage that can be supplied to give the best responses (negative response) due to attenuation of incident light at 0-180^o, and a (+)ve response due to light scattering at ±90^o(reflection from crystal surfaces and it’s shadow). Figure -7A, B shows the effect of variation of light intensity on attenuation of light, scattering light at ±90^o and the algebraic sum of them.

Table 7-Effect of incident light intensity on the measurement of attenuation of incident light (turbidity) as well as reflection of light at two opposite positions and algebraic sum of them.

Incident light intensity (Volt)	Type of measurement $\bar{y}_i \pm t_{0.05/2,\, n-1}\ \sigma_{n-1}/\sqrt{n}$ (n=3) (mV)			
	Attenuation of incident light $T_{(0-180^o)}$	Scattering of light (L) N_L (+90°)	Scattering of light (R) N_R (-90°)	Algebraic sum of the scattering of light $N_{(L+R)}$ (±90°)
0.49	224±3.025	20±1.345	4±0.000	8±0.000
0.66	304±2.924	20±1.007	6±0.000	15±1.384
0.74	312±1.051	32±1.872	6±0.000	16±1.057
0.88	336±1.962	24±2.863	8±0.372	20±2.871
0.97	312±1.489	36±3.621	8±0.000	22±3.164
1.01	320±0.000	40±2.675	8±0.567	24±2.563
1.11	340±1.327	46±1.345	9±0.965	32±0.942
1.15	340±0.985	60±1.562	10±0.872	36±0.543
1.22	280±2.452	80±3.675	12±1.855	40±2.341
1.34	278±3.784	96±2.904	12±2.342	60±2.726
1.43	280±2.675	120±0.875	12±0.758	72±1.435
1.52	276±1.462	133±0.000	20±0.000	80±0.993
1.63	284±1.435	252±0.563	24±0.000	100±0.873
1.74	300±0.673	216±0.982	32±0.699	128±1.008
1.95	292±1.391	296±1.136	40±0.984	168±1.231

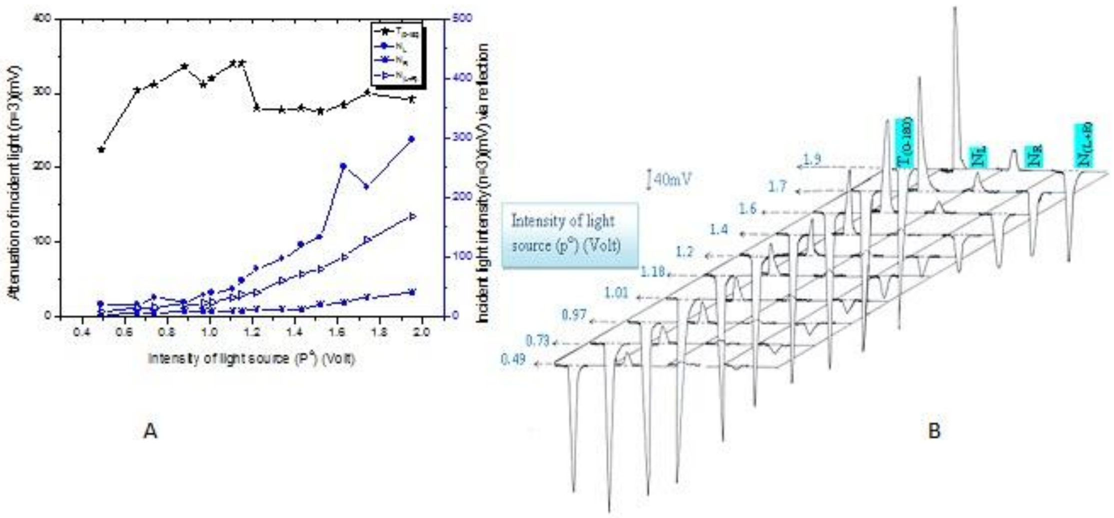


Figure 7- Effect of incident light intensity on; (A) Attenuation of incident light, scattering of light at two opposite directions and the algebraic sum of them, (B) Response profiles.

Stop- Go - Flow Technique for Determination of Hydrogen Peroxide by System Cr (III) - OH^- H_2O_2 - Ba (II)

Using the same chemical and physical parameters for the continuous flow injection mode, the stop – go - flow analysis was studied by varying the time interval of passing the sample section from the injection valve to the oxidation coil. From 0.0-5.0 min were studied. 1.0 min was chosen while gave a good the sensitivity and repeatability of responses. Figure-8 represents the data obtained.

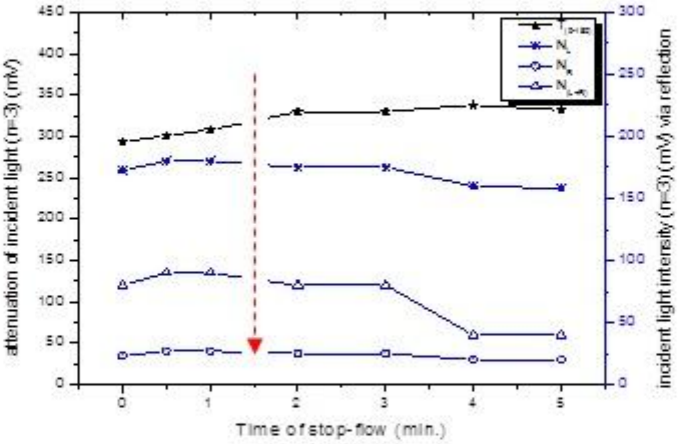


Figure 8- Effect of stop-go mode on attenuation of incident light, scattering of light at two opposite directions and the algebraic sum of them (two Y- axis were used, drawing for 0-180° signal, ±90° and algebraic sum).

Calibration Curves and Statistical Parameters

Using the established optimum chemical and physical parameters, the calibration curves of continuous flow injection analysis and stop- flow via attenuation of incident light, scattering of light at two opposite positions method were constructed. A series 0.25-50 mmol L^{-1} H_2O_2 solution were prepared. The results obtained were tabulated in Table-8. It was noticed that at CFIA method, the attenuation of incident light measurement was most important at low concentration so that its range of calibration carve from 0. 5 mmol.L^{-1} to 25 mmol L^{-1} was achieved because at lower concentration the coagulated particles would be formed small while at higher concentration the import of scattering of incident light increased so that the range of calibration carve for $2N_{90\pm}$ from 0.25 mmol L^{-1} to 30 mmolL^{-1} was achieved because at high concentration of H_2O_2, it might lead to coagulate the precipitated particulate and increased interstitial spaces i.e., increase the scattering of incident light. Paired t-test [16,17] was used to compare among attenuation of incident light ($T_{(0-180)}$), scattering of light at +90 direction (N_L) and scattering of light at -90 direction (N_R). The obtained results as shown in Table -9 indicated clearly that there is no significant differences between $T_{(0-180)}$, N_L and N_R at 95% confidence interval as the calculated t-value is less than critical tabulated t-value.

Table 8-Summary of linear regression equations for estimate of H_2O_2 by CFIA and stop - flow method.

Type of method	Type of measured	No. of measurement	Range of $[H_2O_2]$ mmol L^{-1}	y`(mV)=a± S_at+ b± S_bt[x] at confidence level 95%, n-2	r r^2%	t_{tab} at 95% confidence level, n-2	$t_{cal}=\frac{\lvert r\rvert\sqrt{n-2}}{\sqrt{1-r^2}}$
CFIA	$T_{(0-180)}$	10	0.50-25	26.36±5.66+13.30±0.45[x]	0.9992 99.84	2.306<<71.58	
	N_L	11	0.25- 30	25.75±17.05+7.79±1.17[x]	0.9807 96.17	2.262<<15.04	
	N_R	10	0.50-30	4.20±2.61+1.07±0.17[x]	0.9811 96.25	2.306<<14.32	
	$N_{(L+R)}$	10	0.25-25	14.94±9.51+3.79±1.01[x]	0.9655 93.22	2.306<<10.49	
Stop-flow	$T_{(0-180)}$	11	0.25-30	36.51±6.03+13.01±0.41[x]	0.9991 99.82	2.262<<70.72	
	N_L	12	0.25- 40	35.16±23.59+7.30±1.30[x]	0.9693 93.96	2.228<<12.47	
	N_R	12	0.50-50	8.19±3.26+1.09±0.14[x]	0.9832 96.67	2.228<<17.05	
	$N_{(L+R)}$	10	0.25-25	18.59±0.12+4.15±0.85[x]	0.9691 93.91	2.306<<11.11	

y^: Estimated response (mV) for (n=3), [x] : [H_2O_2]. (mmol L^{-1}), r: correlation coefficient, r^2: coefficient of determination, r^2%:linearity percentage,
t_{tab}: $t_{0.05/2,\ n-2}$ at 95% confidence level.

Table 9-Paired t-test results for $T_{(0-180)}$, N_L and N_R to determine of H_2O_2 at range 0.5 - 25.0 mmol L^{-1} on CFIA method using 100 µL sample volume.

Paired samples	No. of measurement	Paired samples correlations		Paired differences				
		Correlation coefficient (r)	Sig. for (r)	$_d\bar{x}$ Mean	$\overline{Sd}$	Paired t-test $\bar{x}_d\sqrt{n}$ / S_d	df (n-1)	Sig. (2-tailed)
Pair1: turbidity - scattering left	9	0.974	0.00	49	51.68	2.845	8	0.022
Pair 2: turbidity - scattering right	9	0.974	0.00	137	108.41	3.791	8	0.005
Pair 3: scattering left-scattering right	9	0.978	0.00	88	61.15	4.317	8	0.003

$\overline{Sd}$: Different standard deviation, df: degree of freedom

From the results tabulated in Table-9 a hypothesis can be estimated as follow:
Null hypotheses: There is no significant difference between the means obtained in all measured data i.e. $T_{(0-180)}$, $N_{L(0-90)}$,$N_{R(0-90)}$

i. e. H_o: $\mu_T = \mu_{NR} = \mu_{NL}$

Against;
Alternative hypothesis: There is a significant difference between the means of turbidity, scattering of light N_R, and scattering of light at N_L.

i.e. Alternative
A: $\mu_T \neq \mu_{NR} \neq \mu_{NL}$

At a significance level of α= 0.05 (confidence of 95%) any value <0.05 will reject Null hypothesis in favour of Alternative hypothesis any value of significant >0.05 will accept Null hypothesis and reject the alternative hypothesis.
Since all values obtained are < 0.05; Null hypothesis will be rejected and will accept the alternative hypothesis. These mean that there is a significant difference between the measurements of the mean of the three methods used i.e. turbidity measurements, and the scattering of light at two opposite ±90^o.
This is clearly indicated in column no. 4 and 9 in Table-9:

Note: since the incident light is constant therefore any change in turbidity measurement will definitely affect on the reflected light (+90^o) as well as shadow light effect (-90^o). This can be also noticed by excellent correction coefficient of all calibration graphs obtained i.e. a close linear relationship is obtained in the increase of turbidity combined with a decrease in scattered light effect.

To know the relation that binds all these three measurements (N_{L+R} was not taken into consideration due to it is an algebraic sum). Results of stop - and –go mode was subjected to F- test.

A summary of these results is tabulated in Table -10A. From the ANOVA- treatments, it was noticed that there is a significant difference between N_R, N_L and $T_{0-180}{}^o$ at α < 0.05 was obtained. But it difficult to decide which method the difference comes from, and to know or specify where is the difference. Therefore another test should be carried out i.e.; Tukey & Scheffe test which indicate as shown in Table -10B column no.6 shows that 0.353 which is > than 0.05 i.e., no significant difference between 1&2 .While there a significant difference (0.002< 0.05) between 1&3 i.e., turbidity and N_R (scattered light at -90^o) while It was very difficult to decide whether N_L & N_R differ significantly or not as the value of test of significant was (α=0.05).

Table 10A- ANOVA results of stop- flow method for determination of H_2O_2 to range 0.5 - 25.0 mmol L^{-1}.

Type of test	Sum of Squares (SSq)	Degree of freedom (Df)	Mean Square (MSq)	F -statistic	Significant
Between groups	139590.600	2	69795.300	7.726	0.002<0.05
Within group	243916.100	27	9033.930		
Total	383506.700	29	--		

Table 10B-Post Hoc test to know where is significance difference between three methods.

Type of test	**(I) measurements**	**(J) measurements**	**Mean difference (I-J)**	**Std-Error**	**Sig.**
Tukey HSD	1	2 3	59.70000 165.00000	42.50630 42.50630	0.353 0.002
	2	1 3	-59.70000 105.30000	42.50630 42.50630	0.353 0.050
	3	1 2	-165.00000 -105.30000	42.50630 42.50630	0.002 0.050
Scheffe	1	2 3	59.70000 165.00000	42.50630 42.50630	0.386 0.003
	2	1 3	-59.70000 105.30000	42.50630 42.50630	0.386 0.063
	3	1 2	-165.00000 -105.30000	42.50630 42.50630	0.003 0.063

(1): Turbidity measurement (T_{0-180^o}), (2): Scattering at +90° (N_L),
(3): Scattering at -90° (N_R), HSD: Honestly Significant Difference.

Limit of Detection (L.O.D)

A study was carried out to determine the limit of detection of H_2O_2 via successive gradual dilution of the minimum concentration in the linear range which was 0.5mmol L^{-1}, a limit of detection was 0.68 µg /injected sample volume (100µL). Limit of detection for H_2O_2 was calculated through three methods as tabulated in Table-11.

Table 11-Limit of detection H_2O_2 at optimum parameters depend on $T_{(0-180)}$.

Theoretical based on the value of slope $X=3S_B$/slope	Theoretical based on the linear equation $\acute{Y}=Y_B+3S_B$	Practically based on gradual dilution for the minimum concentration
7.67 µg/sample	3.77 µg/sample	0.68 µg/sample

X: value of L.O.D based on slope, S_B: Standard deviation of blank, Y_B: Average response for blank.

Repeatability

The repeatability of measurements and the efficiency of homemade Ayah $4S_W$-3D-T_{180^o} -$2N_{90^o}$ - Solar - CFI Analyser were studied at specified or constant concentrations of hydrogen peroxide (mainly two concentrations were used) using the optimum parameters. Repeated measurements for six successive injections were measured and the obtained results are tabulated in Table-12. It can be seen that the value of the percentage relative standard deviation was less than 2% indicating a reliable measurement can be achieved using this method. Figure-9 is shown a response profile of repeatability at 5 and 15mmol.L^{-1} of H_2O_2 respectively.

Table 12-Repeatability of H_2O_2 at optimum parameters by CFIA- method.

$[H_2O_2]$ mmol.L^{-1}	Type of measurement	Average response $\bar{y}_i$(mV) (n=6)	σ_{n-1}	R.S.D%	Confidence interval of the average response (95% confidence) $\bar{y}_i \pm t_{0.05/2,\ n-1}\ \sigma_{n-1}/\sqrt{n}$
5	$T_{(0-180)}$	92	0.321	0.348	92±0.337
	N_L	92	1.225	1.332	92±1.286
	N_R	9	0.139	1.544	9±0.206
	$N_{(L+R)}$	42	0.291	0.693	42±0.305
15	$T_{(0-180)}$	226	0.333	0.147	226±0.350
	N_L	150	0.952	0.635	150±0.999
	N_R	21	0.286	1.362	21±0.425
	$N_{(L+R)}$	70	1.321	1.887	70±1.387

$t_{0.05/2,\ n-1} = 2.571$, n=6.

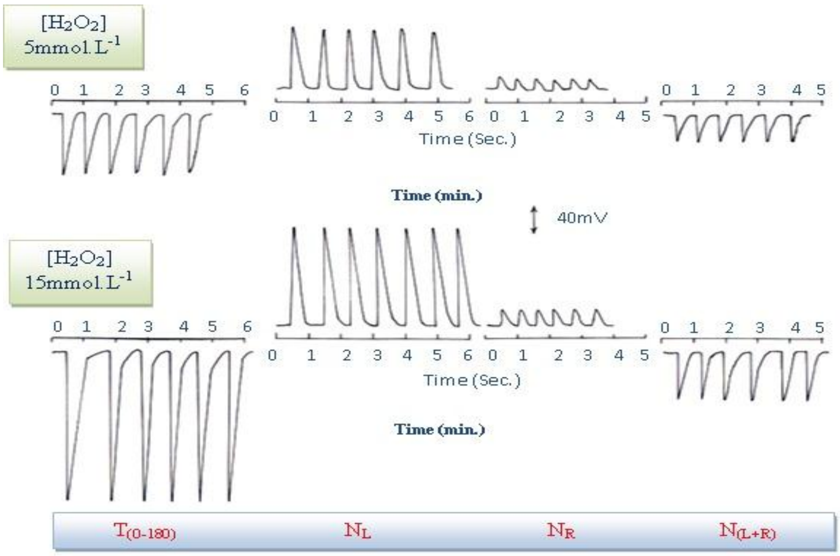


Figure 9-Response profiles of repeatability of attenuation of incident light, scattering of light at two opposite directions and the algebraic sum of them via reflection by CFIA method.

Analysis of Pharmaceutical Preparations

The CFIA and stop- go method via turbidity ($T_{(0-180)}$) and scattered light at two opposite positions ($2N_{\pm 90^o}$) method using Ayah $4S_W$-3D-T_{180^o} -$2N_{90^o}$ -Solar - CFI Analyser achieved in this work was used for the analysis of H_2O_2 in three different of pharmaceutical preparation (Baghdad company, Al-Amire company and Al- Areje company) have 25% , 20% and 7% concentration respectively to evaluate the methods for estimating hydrogen peroxide . After standardization with $KMnO_4$ solution, the concentration of those samples obtained as 9.45%, 1.74% and 1.24% respectively, these different on concentration may be bad storage or closures is not tight for these samples. The data practical concentration of H_2O_2 at $T_{(0-180)}$, scattered light at two opposite positions ($2N_{\pm 90^o}$) and outcome of them by CFIA method and stop –go method are tabulated in Table-13, results data is shown efficiency of methods for determination of hydrogen peroxide.

Table 13-Results of hydrogen peroxide determination in pharmaceutical samples by CFI and stop flow method.

No. of sample	Company, Country, Percentage%, $[H_2O_2]$ mol L^{-1}	percentage% $[H_2O_2]$ mol L^{-1} After standardization	Volume draw (mL) to prepare 20 mmol L^{-1} H_2O_2 in 100mL	Type of measurement	Average response ȳi(mV) (n=3)	Practical $[H_2O_2]$ mmol L^{-1} (n=3)	Practical $[H_2O_2]$ mol L^{-1} and percentage at origin sample	Efficiency of determination
					CFI Stop-go	CFI Stop-go	CFI Stop-go	CFI Stop-go
1	Baghdad company (Iraq) (25%) 7.35 mol L^{-1}	9.45% 2.78	0.72	$T_{(0-180)}$	290 295	19.80 19.88	2.75 9.45%	98.94%
				N_L	176 173	19.47 18.17		
				N_R	25 28	19.47 18.86	2.76 9.39%	99.34%
				$N_{(L+R)}$	88 99.2	19.45 19.45		
2	Al-Amire company (Syria) (20%) 5.88 mol L^{-1}	1.74% 0.51	3.92	$T_{(0-180)}$	288 291	19.79 19.59	0.50 1.71%	98.03%
				N_L	175 173	19.16 18.87		
				N_R	24.6 29	19.09 19.07	0.49 1.69%	97.12%
				$N_{(L+R)}$	89 99	19.51 19.39		
3	Al- Areje company	1.24% 0.37	5.40	$T_{(0-180)}$	281 288	19.15 19.32	0.355 1.20%	95.95%

	(Iraq) (7%) 2.06 mol L^{-1}			N_L	174 170	19.03 18.87	0.358 1.22%	98.13%
				N_R	24 28.9	18.54 19.00		
				$N_{(L+R)}$	87 99	18.98 19.39		

Statistical Treatment of Analytical Data

Two way ANOVA (F-test) treatment [18, 19] was used for the results obtained using both method (CFIA & stop-go). Table-14A shows three types of measurements (T_{0-180}, N_L, N_R). The ANOVA test was carried out at α=0.05 (95% confidence interval), Table -14B shows the summary of ANOVA test at 95% confidence interval which shown that;

Table 14A-Summary of results for measuring samples in three type of measurement and two methods (CFI and Stop-Go).

Type of measurement	Type of method	
	Practical $[H_2O_2]$ mmol L^{-1}, (n=3)	
	CFI	Stop-go
$T_{(0-180)}$	19.80, 19.72, 19.15	19.88, 19.59, 19.32
N_L	19.28,19.26, 19.03	18.87, 18.87, 18.87
N_R	19.47, 19.09,18.54	18.16, 19.07, 19.00

*Theoretical $[H_2O_2]$ = 20 mmol L^{-1}, n: number of samples

Table 14B-Summary of ANOVA test for all methods & measurements.

Source	Sum of Squares (SSq)	Degree of freedom (df)	Mean Square (MSq)	F - statistic	Significant
Corrected Model	1.865	5	0.373	3.222	0.045
Method of measurements	0.144	1	0.144	1.244	0.287
Type of measurements (Turbidity or scattering left and right)	1.614	2	0.807	6.967	0.010
Method of measurement × Type of measurements (Turbidity or scattering left and right)	0.108	2	0.054	0.466	0.639
Error	1.390	12	0.116	--	--
Corrected total	3.255	17	--	--	--

Corrected model: it is given overview where equal to 0.045 which is nearest to 0.05, i.e. don't have detected for the methods found significant different between CFIA and stop go method which used to measure $T_{(0-180)}$, N_L and N_R .

While method of measurements given no significant difference between CFIA and stop - go methods. From type of measurements we can notice that significant difference exist between CFIA method and stop- go method.

The relationship between the two method with type of measurement ($T_{(0-180)}$, N_L and N_R) don't have significant different where sig. value (0.639) is larger than 0.05.

The conclusion that we have six ways for the treatment of results for the three samples and we can used anyone from methods (CFIA and stop- go) at the three type of measurements ($T_{(0-180)}$, N_L and N_R) and depend on it to prepare the calibration carve as well as treatment the three samples. The post-Hoc test calculated to known the value of significant or the details of relationship between any types of measurement with other, as shown in Table-14C.

Table 14C- Summary of Post Host Test ANOVA test of multiple comparisons of all methods &measurement.

Type of test	(I) Type of measurements (Turbidity or scattering left and right)	(J) Type of measurements (Turbidity or scattering left and right)	Sig.
Tukey HSD	1	2 3	0.035 0.011
	2	1 3	0.035 0.803
	3	1 2	0.011 0.803
Scheffe	1	2 3	0.044 0.015
	2	1 3	0.044 0.819
	3	1 2	0.015 0.819

(1): Turbidity measurement ($T_{0-180}{}^{o}$), (2): Scattering at +90^{o} (N_L),
(3): Scattering at -90^{o} (N_R), HSD: Honestly Significant Difference.

Conclusion

The proposed indirect FIA method is a simple, rapid and sensitive for the determination of hydrogen peroxide based on oxidation of Cr (III) ion by hydrogen peroxide in basic medium to form chromate ion which react with Ba (II) ion to produce a yellow precipitate ($BaCrO_4$). The precipitate is measured via the attenuation of incident light and it's scattering in two opposite directions also the algebraic sum of them by using Ayah 4S_W - 3D -$T_{180}{}^{o}$ - 2$N_{90}{}^{o}$ - Solar - CFI Analyser. An alternative analytical method is found through this research work which was based on simple parameter conditions.

Acknowledgement:

I would like to express my deepest gratitude to Prof. Dr. Issam M.A.Shakir Al-Hashimi for his appreciable advice, important comments, support and encouragement.

References

1. Jones, C. W. and Clark, J. H. **1999**. *Applications of Hydrogen Peroxide and Derivatives*, 1st Ed. Royal Society of Chemistry, New York.
2. Salter, H. L. **2012**. *Gateways, the Story of Laporte 1888 -1988*, 1st Ed. Evonik Industries, USA.
3. Wolffenstein, R. **2006**. Reports of the German Chemical Society, Concentration and distillation of hydrogen peroxide, *European Journal inorganic chemistry*, 27 (3), pp: 3307–3312.
4. Riley, **1963**. *Handbook of preparative inorganic chemistry*, 1, 2nd Ed., N.Y.: Academic Press, New York.
5. Grant, W. M. **1974**. *Toxicology of the Eye; Drugs, chemicals, plants, venoms*, 2nd Ed., C. C. Thomas, Springfield, Illinois.
6. Deichmann, W. B. and Gerarde, H. W. **1969**. *Toxicology of Drugs and Chemicals*, Academic press, New York.
7. Chai, Q. Y.; Hou, Q. X., Luo Q. and Zhu J. Y. **2004** Rapid determination of hydrogen peroxide in the wood pulp bleaching stream by a dual- wavelenght spectroscopic method, *Anal. Chim. Acta*, 507, pp:281-284.
8. Lin, K., Tsai, T., and Chen, S. **2010**. Performing enzyme-free H_2O_2 biosensor and simultaneous determination for AA, DA, and UA by MWCNT–PEDOT film, *Biosensors and Bioelectronics*, 26(2), pp:608–614.
9. Li, Y. **1996**. Biological properties of peroxide- containing tooth whiteners, *Food Chem. Toxical*, 34, pp: 887-904.
10. Hill, C. N. **2001**. *A Vertical Empire: The History of the UK Rocket and Space Programme, 1950–1971*, Imperial College Press, New York.
11. Kumar, S. A., Chen S. L. and Chen, S. M. **2010**. Electrochemical sensing of H_2O_2 at Flavin Adenine Dinucleotide/Chitosan/CNT Nanocomposite Modified Electrode, *Electrochemical and Sold-State Letters*, 13(10), pp:83-86.

12. Thangamuthu, R.; Pan Y. and Chen, S., **2011,** Electro catalytic reduction of hydrogen peroxide and its determination in antiseptic and soft –glass cleaning solution at phosphotungsate-doped-glutaraldehyde-cross-linked poly-L-lysine film electrodes, *Sensors and Actuators B*, 151, pp:377-383.

13. Shakir, I. M. A. and Turkey, N. S., **2014**, “Ayah $4S_W$-3D-$T_{180}{}^{o}$ -$2N_{90}{}^{o}$ -Solar cell - CFI Analyser”, *Patent* present to Central Organization for Standardization and Quality control – Baghdad- Iraq, G01N21/00.

14. Vogel, A. I., **1979** “*Text Book of Macro and Semimicro Qualitative Inorganic Analysis”*, 5th Ed., Longman, London.

15. Evans, H. and Mcdicken, W. N., **2000**, “*Doppler Ultrasound*”, 2nd Ed., John Wiley and Sons, New York.

16. Miler, J. C. and Miller, J. N., **2010**, “*Statistics for Analytical Chemistry*”, 6nd Ed., Pearson Education Limited, UK.

17. Bluman, A. G., **1997**, “*Elementary Statistics*”, 3rd Ed., WCB/MC Graw-Hill, New York.

18. Jaisingh, L. R., **2000**, “*Statistics for the Utterly Confused*”, 2nd Ed., McGraw-Hill, London.

19. Brink, D., **2010**, “*Essentials of Statistics*”, Ventus Publishing ASP, London.