

**A new Approach for the Turbidimetric Determination of
Cyproheptadine Hydrochloride in Pharmaceutical Preparations by
Using Homemade Linear Array Ayah 6SX1-T-2D Solar cell CFI
Analyser**

**نمط جديد لقياس التعكرية لتقدير سايبروهبتادين هايدروكلوريد في المستحضرات الصيدلانية
باستخدام محلل الحقن الجرياني المستمر Linear Array Ayah 6SX1-T-2D Solar
cell-CFI Analyser
المصنوع محليا**

***Nagam S.Turkey Al-Awadie , Kefah H. Ismael Al-saadi**

***Chemistry department-College of Science-University of Baghdad-
Baghdad-Iraq
البحث مستل**

Abstract

A new turbidimetric – flow injection method is described for the determination of Cyproheptadine hydrochloride(CPH-HCl) in pure and pharmaceutical preparations .The method is characterized by simplicity ,sensitivity and speed ,it is based on formation of ion-pair between CPH-HCl and hexacyanoferrate (III) in aqueous medium to form yellow precipitate .This precipitate was determined using homemade Ayah 6SX1-T-2D Solar cell-CFI Analyser via the reflection of incident light from the surfaces precipitated particles at (0-180°) .Chemical and physical parameters were studied and optimized .The calibration graph was linear in the range of 0.5- 10 mmol.L⁻¹ , with correlation coefficient $r = 0.9785$,while the percentage linearity (% r^2) was %95.75 .The limit of detection (S/N=3) 280.72 ng /100 μ L from the stepwise dilution for the minimum concentration in the calibration graph with RSD lower than %1.5 for 5and 7 mmol.L⁻¹ (eight replicates) concentration of CPH-HCl .The method was successfully applied for the determination of CPH-HCl in three different companies of pharmaceutical drugs . A comparison was made between the newly developed method analysis with classical method (HANNA instrument for turbidity measurement) using standard addition method via the use of t-test and F-test ,it was found that there was no significant difference between two methods at %95 confidence level .

Key word: Cyproheptadine hydrochloride , Turbidity, Flow injection analysis

الخلاصة :

طريقة جديدة لتقدير السايبروهبتادين هايدروكلوريد من خلال اقتران الحقن الجرياني المستمر مع قياس التعكرية في الحالة النقية والمستحضرات الصيدلانية . الطريقة امتازت بالبساطة , الحساسية والسرعة , تستند الطريقة على تكوين مزدوج ايوني بين السايبروهبتادين هايدروكلوريد مع سداسي سيانات الحديد (III) البوتاسيوم في الوسط المائي لينتج راسب اصفر . قدر الراسب باستخدام محلل الحقن الجرياني المصنوع محليا Ayah6SX1-T-2D solar cell عن طريق انعكاس الضوء المسلط عن سطوح دقائق الراسب بزاوية (0-180°) . تم دراسة كافة المتغيرات الكيميائية والفيزيائية . مدى الخطية لمنحني المعايرة تمتد من 0.5-10 مللي مول . لتر⁻¹ بمعامل ارتباط 0.9785 بينما النسبة المئوية للنوعية للخطية 95.75 % . حدود الكشف (S/N=3) 280.72 نانوغرام / 100 مايكرو لتر من التخفيف التدريجي لاقول تركيز في منحني المعايرة , مع انحراف قياسي نسبي مئوي اقل من 1.5 % لتركيز 7 و 5 مللي مول لتر⁻¹ (تكرارية ثمان مرات) من سايبروهبتادين هايدروكلوريد , الطريقة طبقت بنجاح لتعيين السايبروهبتادين هايدروكلوريد في ثلاث شركات مختلفة . اجريت المقارنة بين الطريقة المستحدثة للتحليل والطريقة التقليدية لقياس التعكرية باستخدام الاضافات القياسية بوساطة اختبار t- المزدوج واختبار F , لوحظ انه لا يوجد فرق جوهري بين الطريقتين عند مستوى قناعة 95 % .

مفتاح الكلمات : سايبروهبتادين هايدروكلوريد , التعكرية , التحليل بالحقن الجرياني

Introduction

Cyproheptadine hydrochloride chemically known as 4-(5*H*-dibenzo[*a,d*]cyclohepten-5-ylidene)-1-methylpiperidine hydrochloride, it is a white or slightly yellow, crystalline powder, slightly soluble in water, freely soluble in methanol, sparingly soluble in alcohol. The structural formula of the salt is shown in Figure no.1 [1].

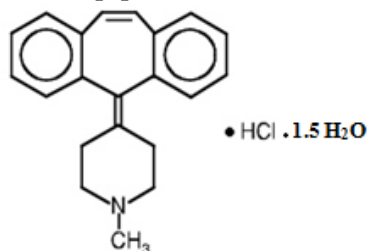


Fig.1: Chemical structure of cyproheptadine hydrochloride

Cyproheptadine hydrochloride is a sedating antihistamine with antimuscarinic, serotonin antagonist, calcium-channel blocking action in pancreatic islet cells and smooth muscle. It is used to treat some hormonal disorders and may also be used for treating side effects of taking antidepressants. Cyproheptadine is also used in clinical and veterinary medicine as an antiserotonergic and anticholinergic effects, as well as to stimulate appetite and weight gain in human and veterinary medicine [2]. Common side effects include drowsiness, dizziness, blurred vision, loss of coordination, upset stomach, constipation, increased appetite, weight gain, thickening of mucus in the nose/throat, or dry mouth/throat/nose [3]. The benefit of use of cyproheptadine in controlling vocalization and disorientation in some cases of baclofen toxicosis and rapidity of weight gain during the refeeding of patients with the restricting diet and relieving depression [4]. Various techniques have been developed for the assay of CPH-HCl in body fluids and pharmaceuticals. Spectrophotometry and turbidimetry [5], titrimetric [6] spectrophotometric [7-10], spectrofluorimetric [11], liquid-liquid microextraction coupled with high performance liquid chromatography [12,13], high performance liquid chromatography and chemometric [14] and liquid chromatographic-tandem mass spectrometric (LC-MS/MS) [15]. Many researchers have used turbidimetric-flow injection for determination of ions and drugs such as hydronium ion [16], chlorpromazine hydrochloride [17], cadmium(II) [18] and amiloride [19].

The aim of this work is to develop a simple and sensitive turbidimetric flow injection method with the use of Ayah 6SX1-T-2D Solar cell-CFIA [20] which supplied with linear array of six snow white light emitting diode as a source and two solar cells as a detector for the determination of cyproheptadine hydrochloride. The method is based on the formation of an ion-pair complex of CPH-HCl with hexacyanoferrate(III) in aqueous medium. Turbidity was measured via the reflection of incident light from the surfaces of solid particulate at 0-180°.

Experimental

Reagents and chemicals

All chemicals were used of analytical-reagent grade while distilled water was used to prepare the solution. A standard solution of Cyproheptadine hydrochloride ($C_{21}H_{22}ClN \cdot 1.5 H_2O$, M.Wt 350.9 $g \cdot mol^{-1}$, SDI, 0.05 $mol \cdot L^{-1}$) was prepared by dissolving 4.3863 g in 250 ml methanol. A stock solution (0.1 $mol \cdot L^{-1}$) of potassium hexacyanoferrate $K_3[Fe(CN)_6]$ (M.Wt 329.26 $g \cdot mol^{-1}$, Fluka) was prepared by dissolving 8.2315 g in 250 ml of distilled water. A 1 $mol \cdot L^{-1}$ of sulfuric acid solution (96% w/w, 1.84 $g \cdot ml^{-1}$, BDH) was prepared by pipetting 14 ml of concentrated sulfuric acid and dilute to 250 ml volumetric flask. A 1 $mol \cdot L^{-1}$ of hydrochloric acid solution (35% w/w, 1.19 $g \cdot ml^{-1}$, BDH) were prepared by pipetting 22 ml of concentrated hydrochloric acid and completed the volume with distilled water in 250 mL. A 1 $mol \cdot L^{-1}$ of nitric acid solution (70% w/w, 1.42 $g \cdot ml^{-1}$

¹,BDH) was prepared by pipetting 16 mL of concentrated nitric acid and completed the volume with distilled water to 250 mL. 1 mol.L⁻¹ acetic acid solution (99.5% w/w, 1.05 g.mL⁻¹, BDH) was prepared by pipetting 15 mL of concentrated acetic acid and completed the volume with distilled water to 250 mL. Each acid was standardized against standard solution of 1 mol.L⁻¹ from Na₂CO₃ (BDH, 105.99 g.mol⁻¹); which prepared by dissolving 26.50 g in 250 mL distilled water.

Sample preparation

Twenty five tablets were weighted, crushed and grinded. The tablets containing 4 mg of cyproheptadine hydrochloride for (IPI- Periahist, Medico – Citadine, Dankos- Nebor) were weighted i.e. (2.772, 5.256, 5.711 g) equivalent to 87.725 mg of active ingredient 5 mmol.L⁻¹ respectively. The powder was dissolved in methanol followed by filtration to remove any undissolved residue affecting on the response and complete the volume to 50 mL with methanol.

Apparatus

Peristaltic pump – 2 channels variable speed (Ismatec, Switzerland) and rotary 6-port medium pressure injection valve, (IDEX corporation, USA) with sample loop (0.7 mm i.d. Teflon, different length). The response was measured by a homemade Ayah 6 SX1-T-2D Solar cell-CFI Analyser, which uses six snow white LEDs for irradiation of the flow cell at 2 mm path length. Two solar cells were used as a detector for collecting signals via sample travel for 60 mm length. The readout of the system composed of x-t potentiometric recorder (Kompensograph C-1032) Siemens (Germany) (1-500 volt, 1-500 mV) or digital AVO-meter (auto range) (0-2 volt) (China). Turbidimetric readings under batch conditions were made by HANNA company (U.S.A.). The flow diagram for the determination of cyproheptadine hydrochloride is shown in Figure. 2

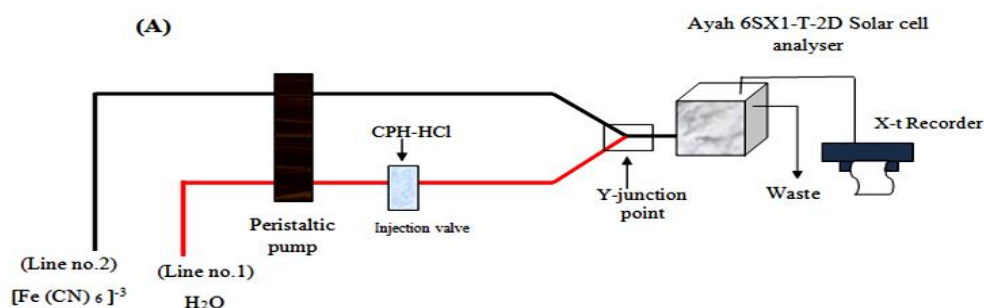
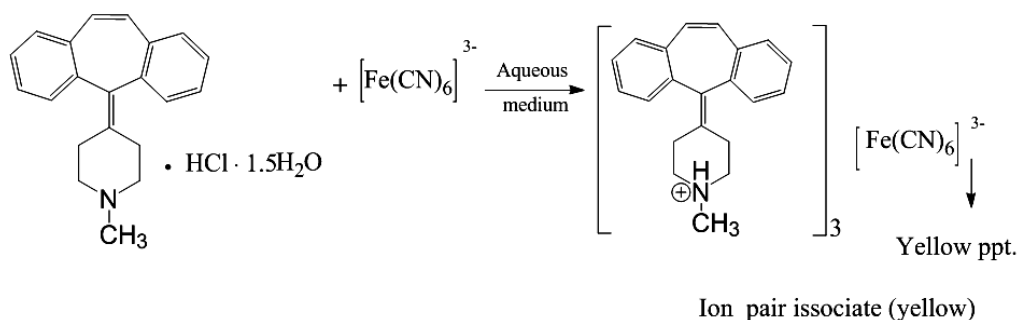


Fig.2 : Flow diagram manifold system used for the determination of Cyproheptadine hydrochloride

Methodology

Reaction of Cyproheptadine hydrochloride with potassium hexacyano ferrate (III) forms a yellow precipitate as an ion pair. The manifold system used as shown in Figure.2 which is composed of two lines. The first line at a flow rate of 1.6 mL.min⁻¹ shows the carrier stream (distilled water) passing through the injection valve to carry the sample segment (Cyproheptadine – HCl, 100 µL of 5 mmol.L⁻¹) to meet the potassium hexacyanoferrate (III) (10 mmol.L⁻¹) carried by the second line (2.3 mL .min⁻¹) at a Y-junction point before it is introduced to the CFI Analyser. Each solution injected was assayed in triplicate. The response profile of which was recorded on x-t potentiometric recorder to measure energy transducer response expressed as peak height in mV by reflection of incident light at 0-180°. The profile was recorded when the applied voltage for the six snow white LEDs was 1.76 volt DC. Scheme.1 shows a proposed mechanism for the reaction CPH-HCl – [Fe(CN)₆]³⁻ in aqueous medium.



Scheme.1: A probable proposed mechanism for the reaction of CPH-HCl with $[\text{Fe}(\text{CN})_6]^{3-}$.

Results and discussion

Study of the optimum parameters

A series of experiments were carried out to establish the optimum condition to be used for the determination of Cyproheptadine hydrochloride using two lines manifold system as shown in Figure.2 . The parameters include chemicals (mainly concentration and type of reaction media) and physical parameters (flow rate ,sample volume , volume of reaction coil , purge time and intensity of incident light) were studied.

Chemical and physical parameters

Effect of variable concentration of $[\text{Fe}(\text{CN})_6]^{3-}$ with different type of reaction media

A series of potassium hexacyano ferrate (III) (0.5 -17) mmol.L^{-1} which is used as a precipitating agent at a flow rate of 2.3 mL.min^{-1} . Different carrier stream were used (hydrochloric acid , sulfuric acid, nitric acid , acetic acid (50 mmol.L^{-1}) in addition to distilled water) at a fixed flow rate of 1.6 mL.min^{-1} in the first line . $104 \mu\text{L}$ sample volume of 5 mmol.L^{-1} CPH-HCl, using 60 second as a purge time (time in seconds to load the manifold with analyte sample) & applied voltage of LEDs was 1.54 volt DC.

It can be seen that increasing reagent concentration up to 10 mmol.L^{-1} will give a better sensitivity expressed as a peak response (height and profile) (Figure.3-A) compared to different acid media used . It might be expected probably that the acids will aid in dissolving or increased solubility of the formed precipitate . Table no.1 is tabulated all the obtained results at a confidence interval of 95 % ($\alpha = 0.05$) . Figure. no.3-A,B shows that the obtained responses in the presence of distilled water indicate that there was no significant differences with responses at different acid media . On that bases distilled water used as the carrier stream .

Table.1: Effect of variable concentration of $[\text{Fe}(\text{CN})_6]^{3-}$ with different type of media on energy transducer response expressed as an average peak heights for the determination of CPH -HCl.

Type of media	$[\text{Fe}(\text{CN})_6]^{3-} \text{ mmol.L}^{-1}$						
	$\bar{y}_i(\text{mV}) \pm t_{0.05/2, n-1} \sigma_{n-1} / \sqrt{n}$						
	0.5	1	5	7	10	15	17
H ₂ O	456 ± 2.43	824 ± 3.06	1428 ± 2.76	1464 ± 3.30	1520 ± 3.80	1528 ± 3.85	1360 ± 4.10
HCl	448 ± 1.69	228 ± 2.81	1144 ± 3.28	1216 ± 3.93	1112 ± 3.85	1416 ± 4.17	1768 ± 4.55
HNO ₃	104 ± 0.97	720 ± 2.61	1244 ± 3.18	1320 ± 3.03	1416 ± 3.53	1496 ± 3.43	1152 ± 3.33
H ₂ SO ₄	104 ± 1.19	376 ± 2.48	1424 ± 3.60	1320 ± 3.28	1488 ± 3.43	1384 ± 4.173	1448 ± 3.58
CH ₃ COOH	280 ± 1.47	816 ± 2.56	1336 ± 4.17	1404 ± 3.53	1496 ± 3.68	1336 ± 3.53	1256 ± 3.93

$\bar{y}_i$: Energy transducer response expressed as an average peak heights in mV for n= 3

$t_{0.05/2, 2} : 4.303$

(A)

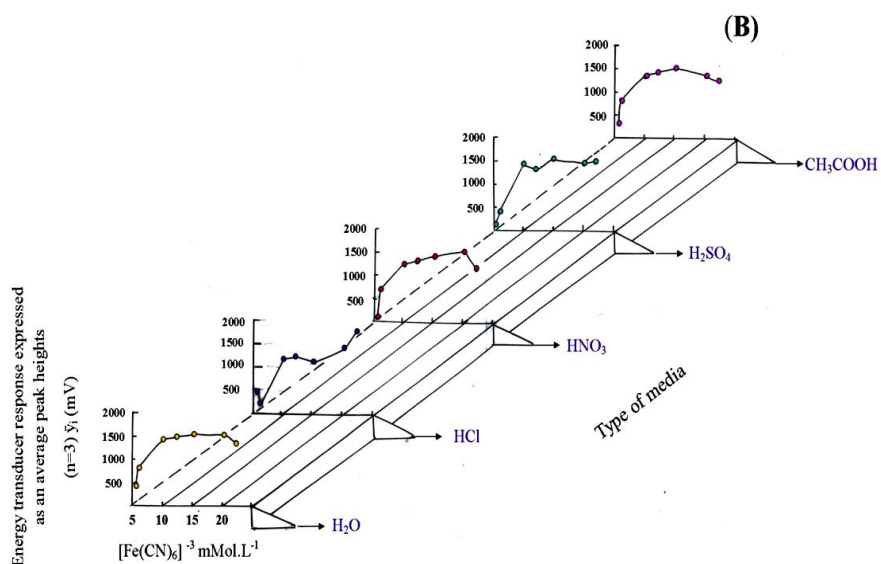
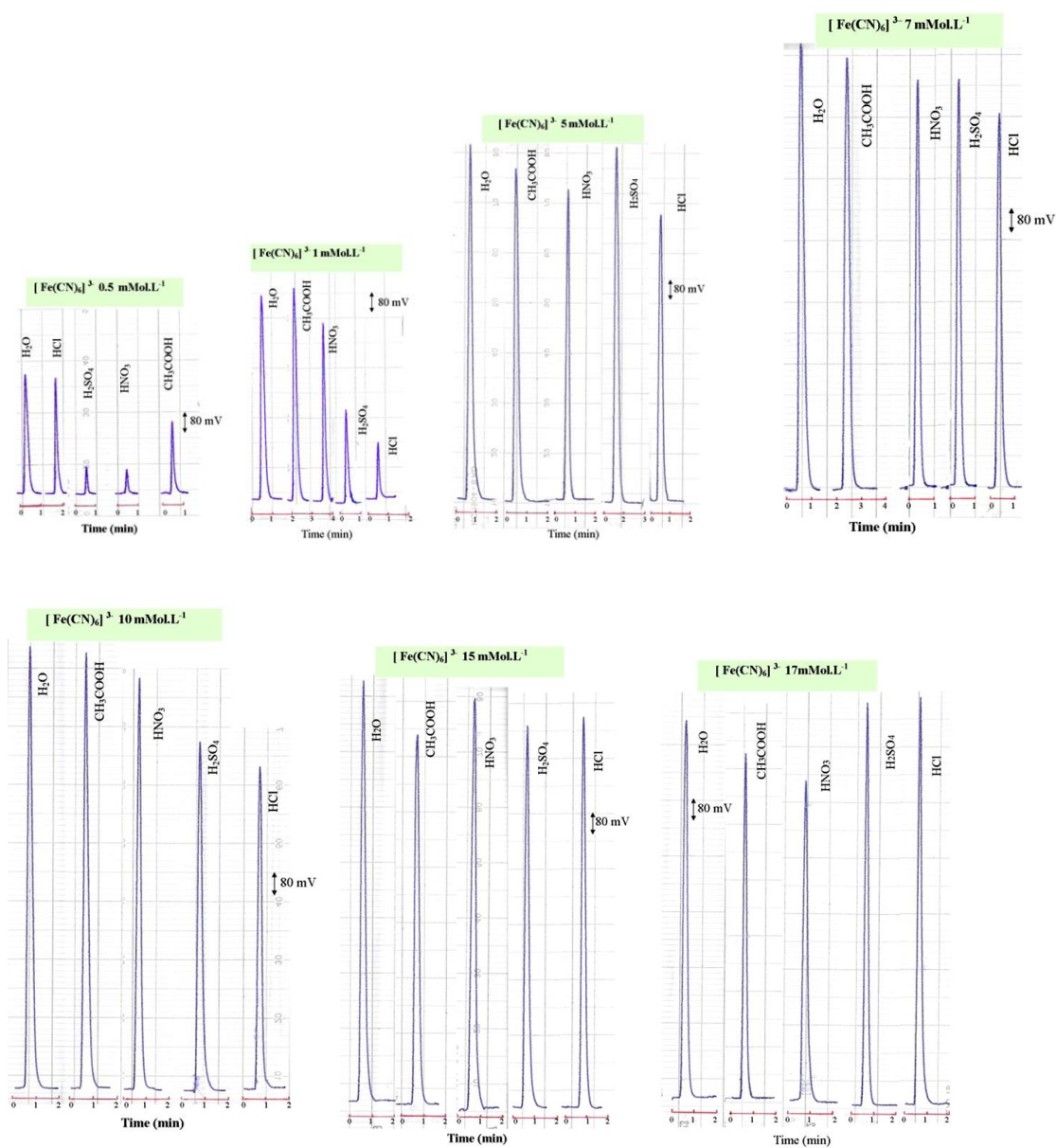


Fig.3: Effect of variable concentration of $[\text{Fe}(\text{CN})_6]^{3-}$ with different type of media on:
(A):Response profile vs.time using Ayah 6SX1-T-2D-Solar cell CFIA, **(B):** Energy transducer response expressed as an average peak heights in mV by reflection of incident light .

Flow rate

Using CPH-HCl(5 mmol.L⁻¹)- $[\text{Fe}(\text{CN})_6]^{3-}$ (10 mmol.L⁻¹) system , 104 μL sample volume , open valve(60 sec) and applied voltage of LEDs was 1.54 volt DC . Different flow rates (0.25-2.4) , (0.5-3.5) mL.min⁻¹ for carrier stream and precipitating agent respectively . Figure.4-A,B shows that at low flow rate there were an increase in peak height up to 0.95,1.2 mL.min⁻¹ for carrier stream(distilled water) and reagent respectively with wider peak width , this might be due to dilution and dispersion , followed by constant and then decrease of peak height at flow rate > 1.6,2.3 mL.min⁻¹.Figure.4-C shows the relation of the contribution of both lines carrier stream and reagent expressed as a ratio of reagent line to carrier line vs. Δt_b or Figure.4-D between energy transducer response with average of flow rate , it was noticed that the ratio between reagent line and carrier line increase with increasing of flow rate and this is due to increase in contribution of the second line (i.e: reagent line) compared with first line (i.e: carrier line) . So the best flow rate for the completion reaction between the CPH-HCl and $[\text{Fe}(\text{CN})_6]^{3-}$ were 1.6 and 2.3 mL.min⁻¹ to obtain a regular response, narrow Δt_B and minimize the consumption of reaction solution . All results tabulated in Table 2.

Pump speed (approximate)	Flow rate ml .min ⁻¹		$\bar{x}$ ml.min ⁻¹	*Ratio	Δt_b (cm)	Energy transducer response expressed as an average peak heights (n=3) $\bar{y}_i$ (mV)	RSD %	Confidence interval at (95%) $\bar{y}_i \pm t_{0.05/2, n-1} \sigma_{n-1}/\sqrt{n}$	Base width Δt_B (sec)	t sec	V_{final} mL	Concentration in mmol.L ⁻¹ at flow cell after dilution	*Df
	Relative contribution (cm)												
	Carrier stream	Reagent											
5	0.25	0.50	0.375	1.31	6.6	1500	0.09	1500 ± 3.28	138	12 6	1.829	0.284	17.61
	2.90	3.80											
10	0.60	0.85	0.725	1.31	6.6	1640	0.11	1640 ± 4.45	96	72	2.424	0.215	23.26
	2.90	3.80											
15	0.95	1.20	1.075	1.31	6.6	1900	0.07	1900 ± 3.28	78	42	2.899	0.179	27.93
	2.90	3.80											
20	1.25	1.80	1.525	1.76	7.8	1640	0.09	1640±3.68	42	30	2.239	0.232	21.55
	2.90	5.10											
25	1.60	2.30	1.950	2.03	8.6	1640	0.09	1640 ±3.78	36	27	2.444	0.213	23.47
	2.90	5.90											
30	1.80	2.60	2.200	2.17	9.0	1320	0.10	1320±3.30	30	24	2.304	0.226	22.12
	2.90	6.30											
35	2.20	3.10	2.650	2.34	9.4	1020	0.16	1020±3.95	21	21	1.959	0.265	18.87
	2.90	6.80											
40	2.40	3.50	2.950	2.66	10.3	1040	0.19	1040±4.80	18	18	1.874	0.277	18.05
	2.90	7.70											

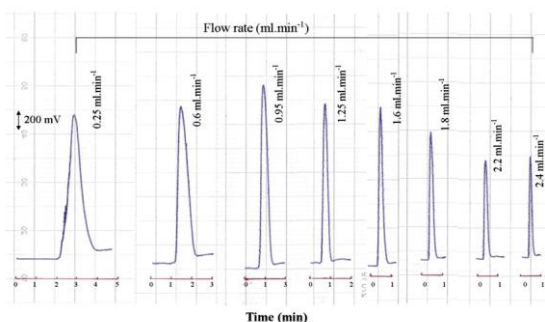
Table .2: Effect of variable flow rate on the measuring of energy transducer response via reflection of incident light for determination of CPH-HCl - $[\text{Fe}(\text{CN})_6]^{3-}$ system .

$\bar{x}$:average of flow rate for both line, *Ratio= contribution of reagent line /contribution of carrier line

Δt_b : Relative output of both line (cm) , V_{final} : Final volume (mL) at variable flow rate

t:Departure time for sample segment from injection valve to measuring cell , *Df : Dilution factor at variable flow rate

(A)



(B)

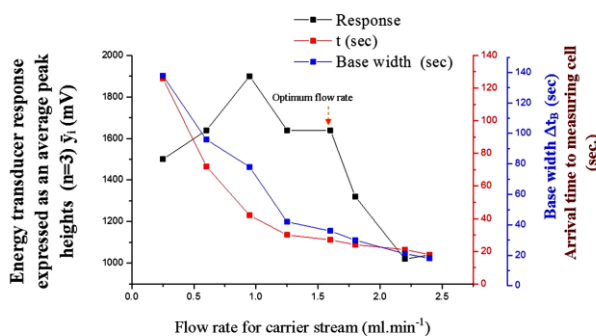
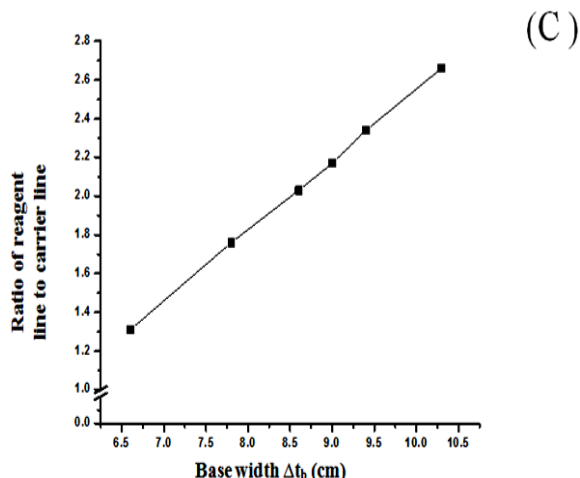


Fig.4: Effect of flow rate on:

(A): Response profile vs. time

(B): Energy transducer response expressed as an average peak heights in mV by reflection of incident light for CPH-HCl-[Fe(CN)₆]³⁻ system, 104 μL sample volume.



(D)

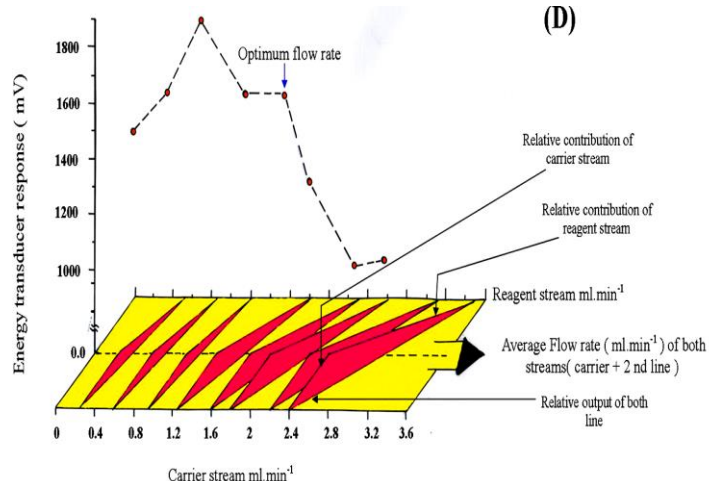


Fig.4 (C): Variation of contribution ratio of line (2) (reagent) to line (1) (carrier) versus base width (Δt_b) at variable flow rate

(D): Plot of energy transducer response vs. average flow rate of both line (carrier + reagent). Indicating at the x-axis the contribution effect of each line also it shows the resultant output.

Effect of different sample volume with variable purge time

Using CPH-HCl (5 mmol.L⁻¹) – [Fe(CN)₆]³⁻ (10 mmol.L⁻¹) system. Flow rate 1.6, 2.3 mL.min⁻¹ for carrier stream (distilled water) and reagent respectively and applied voltage of LEDs was 1.54 volt DC. The injection volume was varied from 70–112 μL using different purge time (2–13 sec) in addition to open valve mode (60 sec). It was noticed (Figure.5-A) that any increase in the sample volume up to 100 μL lead to an increase in the height of responses followed by a minor decrease or being approximately constant response. This might be attributed to elongation of the period in front of the detection point due to enlarged sample volume as well as the weight or size of the formed precipitate particles which might cause a slight delay to this weight. At the same time; purge time was studied. The results obtained were tabulated in Table 3. It can be noticed from the table that there is an increase in the response with increasing the allowed permissible time for the sample injection up to 10 sec, followed by a slight decrease in the response as shown in Figure. 5-A,B.

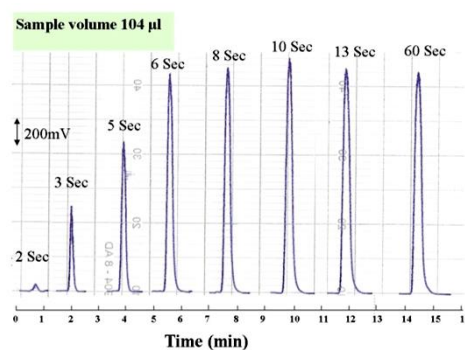
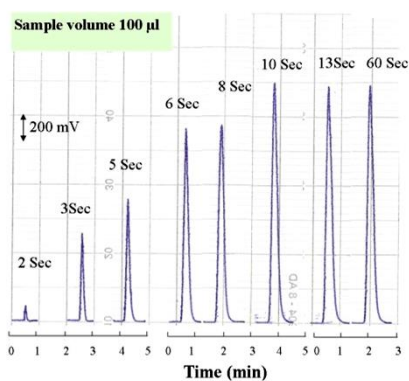
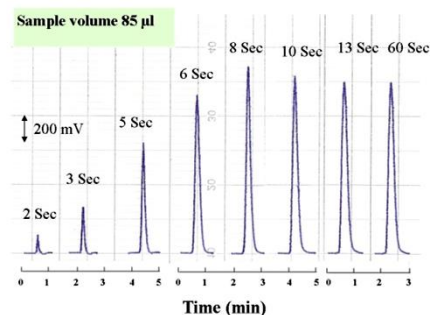
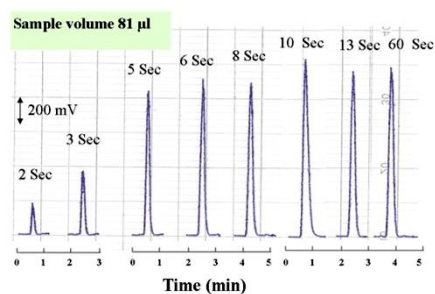
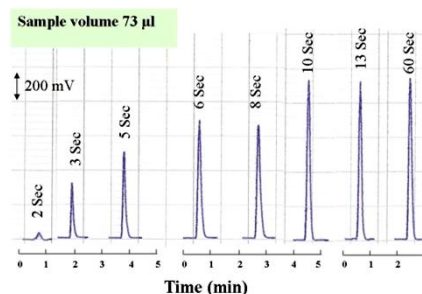
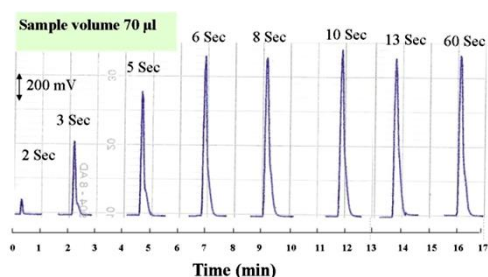
Also, it can be inferred that an increase in the injection time above 10 sec causes a destruction of the flow as a result of elongated period of leaving the injection sample in the injection valve (injection mode) which lead to the slow movement of the precipitate particulate segment in the measuring flow cell for a longer period of time . Therefore, 100 μ L and 10 sec were chosen as an optimum conditions for the future studies.

Table .3 : Effect of different sample volume with variation purge time on energy transducer response for determination of CPH-HCl using Ayah 6SX1-T-2D Solar cell CFIA .

Sample volume (μ l)	Purge time (sec)							
	$\bar{y}_i(\text{mV}) \pm t_{0.05/2, n-1} \sigma_{n-1} / \sqrt{n}$							
	2	3	5	6	8	10	13	Open valve (60 sec)
70	100 $\pm$ 3.03	540 $\pm$ 3.28	900 $\pm$ 3.06	1160 $\pm$ 3.28	1160 $\pm$ 3.93	1220 $\pm$ 3.03	1160 $\pm$ 4.17	1160 $\pm$ 3.28
73	60 $\pm$ 3.03	400 $\pm$ 3.28	620 $\pm$ 3.08	860 $\pm$ 3.28	820 $\pm$ 3.08	1160 $\pm$ 3.28	1140 $\pm$ 2.76	1160 $\pm$ 3.33
81	220 $\pm$ 3.30	460 $\pm$ 3.08	1040 $\pm$ 3.18	1040 $\pm$ 3.03	1020 $\pm$ 3.30	1300 $\pm$ 3.03	1200 $\pm$ 3.08	1220 $\pm$ 3.53
85	140 $\pm$ 3.50	340 $\pm$ 3.53	800 $\pm$ 3.33	1160 $\pm$ 3.53	1360 $\pm$ 3.25	1300 $\pm$ 3.25	1240 $\pm$ 3.50	1240 $\pm$ 3.28
100	100 $\pm$ 3.28	640 $\pm$ 3.25	900 $\pm$ 2.43	1410 $\pm$ 2.43	1440 $\pm$ 2.51	1740 $\pm$ 3.05	1720 $\pm$ 3.00	1700 $\pm$ 3.28
104	50 $\pm$ 2.43	620 $\pm$ 2.36	1100 $\pm$ 2.04	1580 $\pm$ 0.97	1640 $\pm$ 2.46	1710 $\pm$ 2.43	1640 $\pm$ 3.53	1620 $\pm$ 2.76
112	40 $\pm$ 2.34	700 $\pm$ 2.38	840 $\pm$ 2.09	1440 $\pm$ 1.94	1680 $\pm$ 3.06	1680 $\pm$ 3.30	1360 $\pm$ 3.58	1370 $\pm$ 3.28

$\bar{y}_i$: Energy transducer response expressed as an average peak heights in mV for n = 3, $t_{0.05/2, 2}$: 4.303

(A)



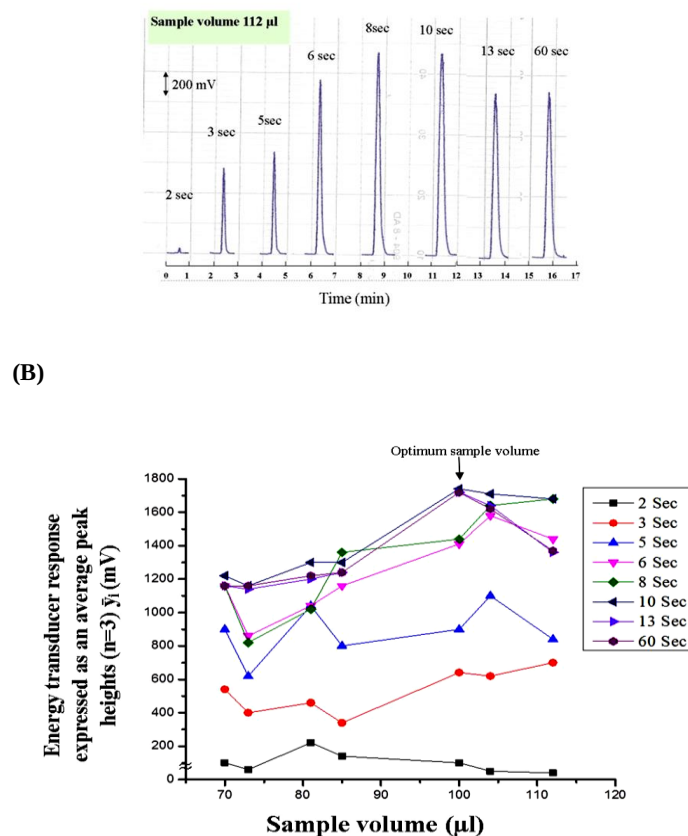


Fig. 5: Effect of different sample volume with variation purge time on

(A): Response vs. time profile

(B): Energy transducer response expressed as an average peak heights (mV) using Ayah 6SX1-T-2D-Solar cell CFIA.

Reaction coil

Variable coil length 0-100 cm was study . These length comprises a volume (0 - 0.785) mL which connected after Y-junction directly in flow system . While keeping all other changeable constant (i.e: CPH-HCl 5 mmol.L⁻¹ , [Fe(CN)₆]³⁻ 10 mmol.L⁻¹ , flow rate (1.6,2.3) mL.min⁻¹ for carrier stream(distilled water) and reagent respectively ,sample volume 100µL , purge time (10 sec) and applied voltage of LEDs was 1.54 volt DC. Figure.6-A,B shows that the increase of coil volume lead to decrease of peak height with increase of base width and departure time for sample segment from injection valve to measuring cell this might be attributed to diffusion &dispersion of precipitate particulate segment due to increase of dispersion regions and mostly lead to accumulation of precipitate particles causing lose of some of the reflecting surface . So, it can be seen clearly that no reaction coil necessary for completion reaction between CPH-HCl and [Fe(CN)₆]³⁻ in aqueous medium. Table4 shows all results .

Table.4: Effect of coil length on energy transducer response expressed as an average beak heights (mV) for determination of CPH-HCl .

Coil length (cm)	Coil volume (mL) $r^2\pi h$, $r=0.5$ mm	Energy transducer response expressed as an average peak heights (n=3) $\bar{y}_i$ (mV)	RSD %	Confidence interval at (95%) $\bar{y}_i \pm t_{0.05/2, n-1} \sigma_{n-1} / \sqrt{n}$	t (sec)	Base width Δt_B (sec)	V_{final} (ml)	Concentration in mmol.L ⁻¹ at flow cell
0	0	1740	0.07	1740 ± 3.06	12	60	4.00	0.125
30	0.235	1500	0.08	1500 ± 2.98	18	84	5.56	0.090
60	0.471	1200	0.09	1200 ± 2.81	30	90	5.95	0.084
100	0.785	1120	0.10	1120 ± 2.68	36	96	6.34	0.079

t : Departure time for sample segment from injection valve to the measuring cell.

V_{final} : Final volume (mL) at flow cell

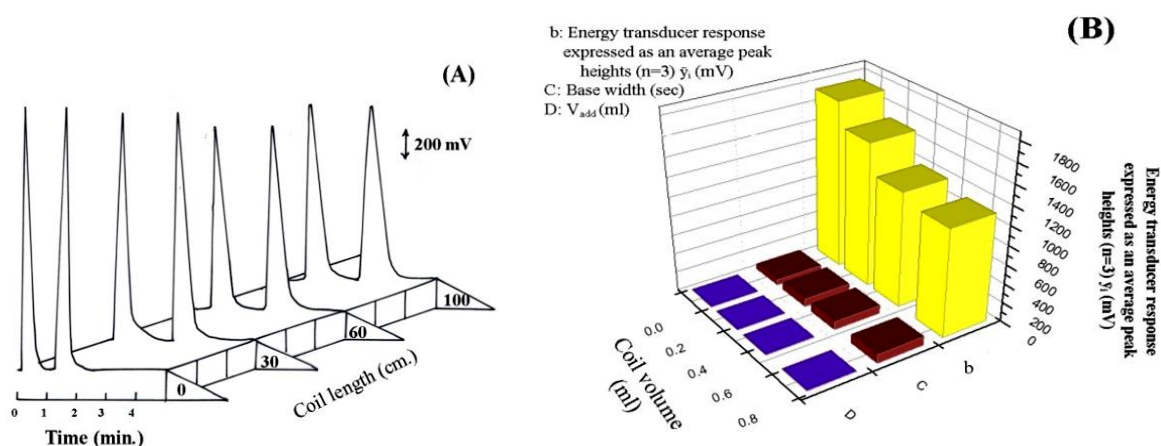


Fig.6: Effect of reaction coil on :

(A): Response profile vs. time

(B): Variation of coil volume on

b: Energy transducer response expressed as an average peak heights in mV

C: Base width (sec.) , D: V_{add} (mL).

Intensity of light

Intensity of light source was studied using CPH-HCl (5 mmol.L⁻¹)-[Fe(CN)₆]³⁻ (10 mmol.L⁻¹) system while keeping all other changeable constant . Variable intensity of light source (0.071-2) volt were used by variation of light intensity knob of Ayah 6SX1-T-2D Solar cell CFIA . Figure. 7 shows that an increase in peak height with increase of intensity; Therefore 1.76 volt selected as the optimum voltage of snow white to preserved the life of LED and it gave the best response compared with a response of 1.9 and 2 volt that shows in Figure.7-A or B . All results tabulated in Table 5 .

Table. 5 : Effect of intensity of light on the measuring of energy transducer response for determination of CPH-HCl using Ayah 6SX1-T-2D Solar cell CFIA.

Applied voltage expressed as intensity of light (Volt)	Energy transducer response expressed as an average peak heights (n=3) $\bar{y}_i$ (mV)	RSD%	Confidence interval at(95%) $\bar{y}_i \pm t_{0.05/2, n-1} \sigma_{n-1} / \sqrt{n}$
0.460	220	0.31	220 ± 1.69
0.609	260	0.12	260 ± 0.79
0.970	940	0.12	940 ± 2.71
1.150	1280	0.08	1280 ± 2.43
1.420	1620	0.11	1620 ± 4.55
1.540	1740	0.07	1740 ± 3.08
1.620	1820	0.09	1820 ± 4.02
1.760	2020	0.07	2020 ± 3.35
1.800	2020	0.07	2020 ± 3.35
1.980	2090	0.06	2090 ± 3.12
2.000	2080	0.06	2080 ± 3.28

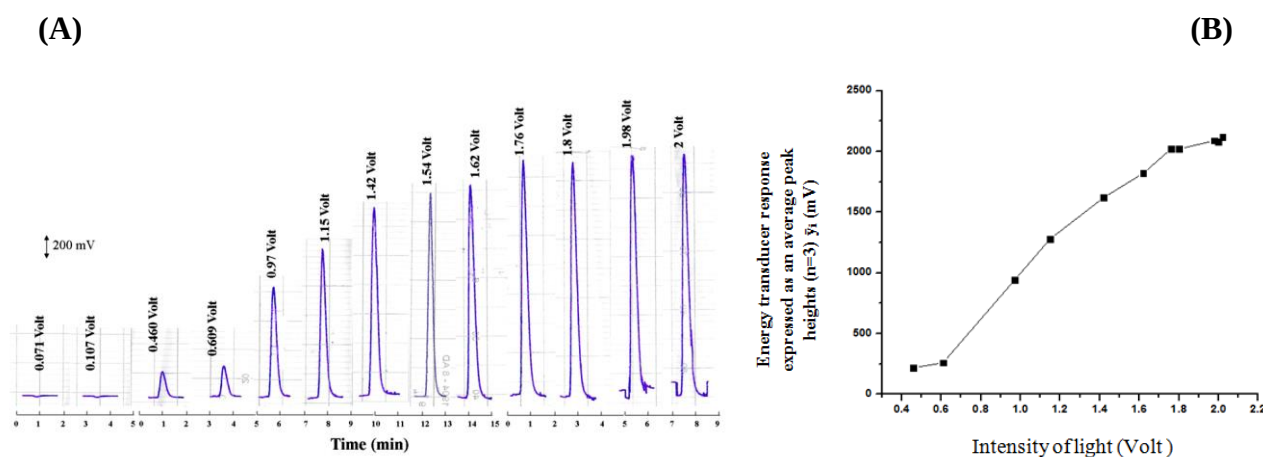


Fig.7: Effect of light intensity on:

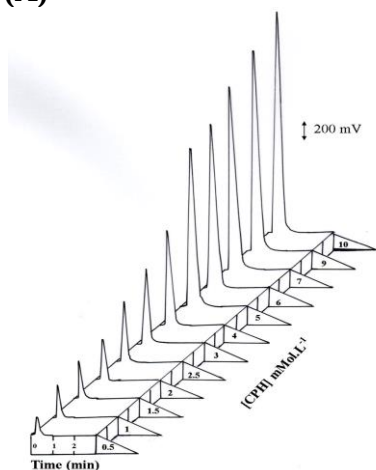
(A): Response profile versus time via the reflection of incident light

(B): Energy transducer response expressed as an average peak heights in mV using 100 µL sample volume .

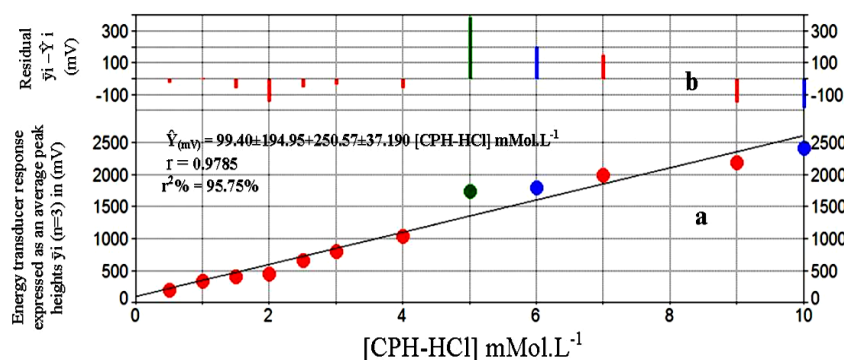
Scatter plot calibration curve

Under the established optimum condition, the calibration curve of continuous flow injection analyses coupled with Ayah 6SX1-T-2D-Solar cell-CFI Analyser via reflection of incident light were estimated .A scatter plot diagram shows that a linear calibration graph rang for the variation of the energy transducer response with CPH-HCl concentration was ranging from 0.5-10 mmol.L⁻¹ with correlation coefficient r:0.9785 as shown in Figure.8- B . Compared with classical method via measurement of turbidity by HANNA instrument .A linearity calibration curve was obtained for the concentration range of 0.5-5 mmol.L⁻¹ of CPH-HCl as shown in Figure.8- C , correlation coefficient was r: 0.9842 and detection limit 100µmol.L⁻¹ . All results tabulated in Table 6.

(A)



(B)



(C)

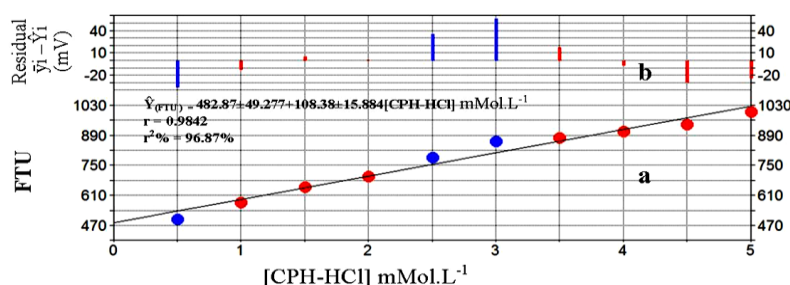


Fig.8: Calibration graph for the variation of CPH-HCl concentration on:

(A): Response profile versus time

(B):a - Energy transducer response expressed by linear equation using Ayah 6SX1-T-2D Solar-CFI Analyser ,b- residual ($\bar{y}_i - \hat{Y}_i$), $\bar{y}_i$: practical value, $\hat{Y}_i$: estimate value.

(C):a- FTU by linear equation using HANNA instrument , b- residual ($\bar{y}_i - \hat{Y}_i$), $\bar{y}_i$: practical value, $\hat{Y}_i$: estimate value.

Table.6: Summary of results for linear regression analysis using Ayah-6SX1-T-2D-Solar cell-CFIA and classical method via measurement turbidity by HANNA instrument using first degree equation.

Ayah 6SX1-T-2D-Solar cell-CFIA					
HANNA instrument					
Measured [CPH-HCl] mMol.L ⁻¹	Range of [CPH-HCl] mMol.L ⁻¹	$\hat{Y}_i = a \pm s_a t + b \pm s_b t [\text{CPH-HCl}]$ at confidence level 95%, n-2	r r ² %r ²	t _{tab} at 95%, n-2	$t_{cal} = \frac{ r \sqrt{n-2}}{\sqrt{1-r^2}}$
0.01-17	0.5-10 n=12	99.40 ± 194.95 + 250.57 ± 37.190[X]	0.9785 0.9575 95.75%		2.228 < 15.011
0.01-6	0.5-5 n=10	482.87 ± 49.277 + 108.38 ± 15.884[X]	0.9842 0.9687 96.87%		2.306 < 15.734

[X]=[CPH-HCl] mMol.L⁻¹, $\hat{Y}$ =Estimate value in mV or FTU = Formazin turbidity unit,
r = Correlation coefficient , r²% = Percentage linearity, r²= Coefficient of determination (C.O.D)

Limit of detection

A study was carried out to calculate the limit of detection of CPH-HCl through two method .Gradual dilution of low concentration in the calibration graph or based on the numerical value of slope . The results tabulated in Table. 7.

Table.7: Limit of detection for CPH-HCl at optimum parameters using 100 μ L as an injection sample.

Practically based on the gradual dilution for the minimum concentration (0.008 mmol.L ⁻¹)	Theoretical based on the value of slope $x=3S_B/\text{slope}$ for n=13
280.72 ng/ 100 μ L Sample	63.0192 ng/100 μ L Sample

x = value of L.O.D. based on slope , S_B = standard deviation of blank repeated for 13 times

Repeatability

The value of the relative standard deviation (RSD%) for CPH-HCl 5 and 7 mmol.L⁻¹ was tabulated in Table. 8 . The percentage relative standard deviation less than 1.5% was obtained indicating a reliable measurement can be achieved using this method . Response profile shows in Figure .9 .

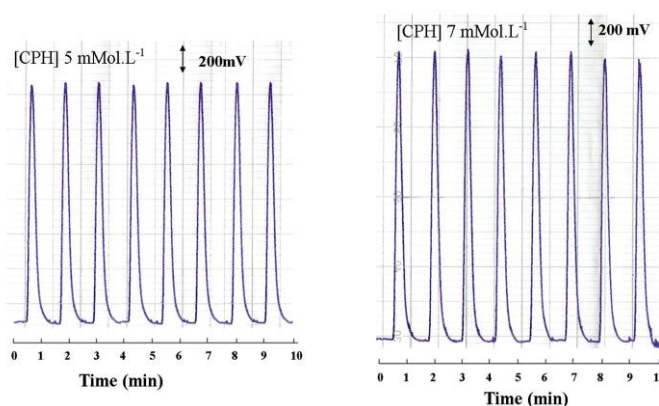


Fig. 9: Response – time profile for eight successive repeated measurements of CPH-HCl concentration (5 mmol.L⁻¹ & 7 mmol.L⁻¹) using Ayah 6SIX1-T-2D Solar cell CFIA .

Table.8: Repeatability of CPH-HCl at optimum parameters with 100 μ l sample volume via reflecting of incident light expressed as an energy transducer response .

[CPH-HCl] mMol.L ⁻¹	Average response $\bar{y}_i$ (mV) $n^*=8$	RSD %	Confidence interval at 95% $\bar{y}_i \pm t_{0.05/2, n-1} \sigma_{n-1} / \sqrt{n}$
5	1723.75	0.69	1723.75 $\pm$ 9.93
7	2070.00	1.03	2070.00 $\pm$ 17.88

n^* =Number of injection

$t_{0.05/2,7}= 2.365$

Treatment of data

Three approaches were made using three different mode of treatment

A- Analyses of variance (ANOVA) 2 way [21,22]

B- Parid t- test of comparison of two method

C-A plot of two different y-axis at constant concentration used through calibration work .

There are the details of this above three approaches and as follows :

A- Analyses of variance (ANOVA) 2 way

Analysis of Table no.6 data using two tailed ,analysis of variance (ANOVA) was conducted based on the assumption of the two effects :

- Variation of method used in analysis
- Variation of concentration

A summary of this results is tabulated in Table.9.

Table. 9: Summary of results using ANOVA test for determination of CPH-HCl-[Fe(CN)₆]³⁻ system of rang 0.5-5 mmol.L⁻¹.

Source of variation	Sum squares (SSq)	Degree of freedom(df)	Mean square (Msq)	F _{cal}	*F _{tab}	Sig
Type of method	333893.500	1	333893.500	27.102	> 8.07	0.001
Variation of concentration	657138.985	7	93876.998	7.620	> 4.99	0.008
Error	86240.000	7	12320.00			
Total	1077272.48515	15				

* $F_{v2}^{v1} = F_7^1 = 8.07$ and $F_7^7 = 4.99$ at 95% confidence level (2-tailed)

From the ANOVA treatment , effect of methods and concentration on the response (measurements), it was found a significant difference between two method at $\alpha = 0.05$, 2 – tailed due to sig. < 0.05 or $F_{tab} < F_{cal}$.

B-Paired t- test of comparison of two method classical method (turbidity measurement) & developed method using Ayah 6SX1-T-2D Solar cell CFIA.

Using paired t-test at $\alpha = 0.05$ (2-tailed) for the comparison of developed method using Ayah 6SX1-T-2D Solar cell CFIA and classical method using HANNA instrument (turbidity measurement) at a range of concentration for a eight variable concentration(0.5-5) mmol.L⁻¹ that represent scatter plot of the data obtained .The summary of results are tabulated in Table.10.

Table. 10: Summary of results using paired t-test for two method .

Type of pair	Mean ($\bar{x}$) mMol.L ⁻¹	N	Std. Deviation	Std.Error Mean*	Correlation (r)	Sig.	Paired differences			
							Mean	t_{cal} = $\bar{x}d\sqrt{n}/std$	t_{tab}	Sig. (2- tailed)
							Std. Deviation	Degree of freedom (df)		
Developed method- Classical method	709.6250	8	496.39383	175.50172	0.924	0.001	-39.12500	-0.322 < 2.365		0.05 <0.757
	748.7500	8	171.90924	60.77909			343.93124	7		

N: no. of measurements (i.e. no. of response) , *SEM = $\frac{\sigma n-1}{\sqrt{N}}$

From the results obtained that were tabulated in Table.10 a hypothesis can be estimated as follow :

Null hypothesis : There is no significant difference between the means for both methods

i.e : $H_0: \mu_{\text{Ayah 6SX1-T-2D Solar cell}} = \mu_{\text{HANNA instrument}}$

Against :

A lternative hypothesis : There is asignificant difference between the mean of classical method and Ayah 6SX1-T-2D Solar cell CFIA

i.e. Alternative $H_1: \mu_{\text{Ayah 6SX1-T-2D Solar cell}} \neq \mu_{\text{HANNA instrument}}$

There is no significant difference between two methods at $\alpha = 0.05$ (2-tailed) due to sig. > 0.05 or $t_{tab} > t_{cal}$. A correlation 0.924 indicate $r^2 = 0.8538$ that show that 85.38% of the data obtained can be explained by the obtained linear regression model equation. So a both methods of analysis are strongly correlated (i.e: 0.924) .

The difference analysis of data between paired t-test and ANOVA-treatments due to :

Paired t-test used to compare two methods, one of them is standard or two results one of them is reference methods , while ANOVA test is applied to more than that .

C-A plot of two different y-axis at constant concentration used through calibration work.

A summary of results for this study is tabulated in Table11-A,B and Figure.10.

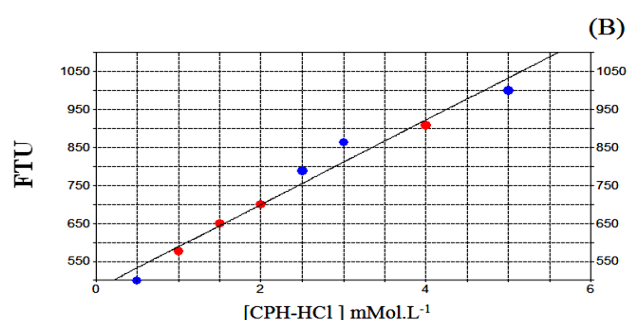
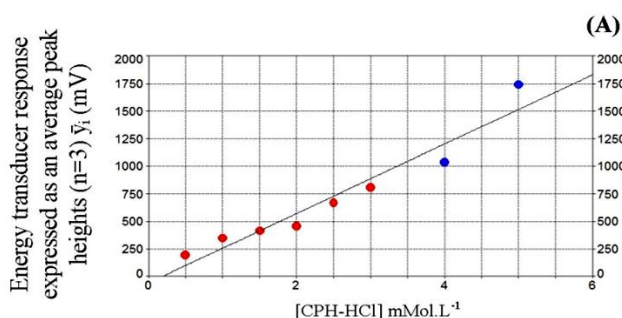
Table. 11-A: Summary of results for two methods showing variation of concentration of CPH-HCl on $\hat{Y}_i$ (y_i predicted) value

[CPH-HCl] mMol.L ⁻¹	Ayah 6SX1-T-2D Solar cell		HANNA instrument	
	Energy transducer response expressed as an average peak heights (n=3) $\bar{y}_i$ (mV)	$\hat{Y}_i$ predicted ($\hat{Y}_i$) mV	Turbidity FTU	$\hat{Y}_i$ predicted ($\hat{Y}_i$) FTU
0.5	198	99.682	500	533.378
1.0	350	257.087	578	588.958
1.5	415	414.491	650	644.538
2.0	456	571.896	700	700.118
2.5	668	729.301	789	755.698
3.0	810	886.705	864	811.277
4.0	1040	1201.515	909	922.437
5.0	1740	1516.324	1000	1033.597

Table. 11-B: Summary of results for linear regression analysis using Ayah-6SX1-T-2D-Solar cell-CFIA and classical method via measurement turbidity by HANNA instrument using first degree equation.

Range of [CPH-HCl]mMol.L ⁻¹	$\hat{Y}_i = a \pm s_a + b \pm s_b t$ [CPH-HCl] at confidence level 95%, n-2	r r ² % r ²
0.5-5	$\hat{Y}_{(mV)} = -57.72 \pm 240.03 + 314.81 \pm 85.03 [X]$	0.9653 0.9319 %93.19
	$\hat{Y}_{(FTU)} = 477.80 \pm 56.281 + 111.16 \pm 19.94 [X]$	0.9843 0.9688 % 96.88

[X] : [CPH-HCl] mMol.L⁻¹



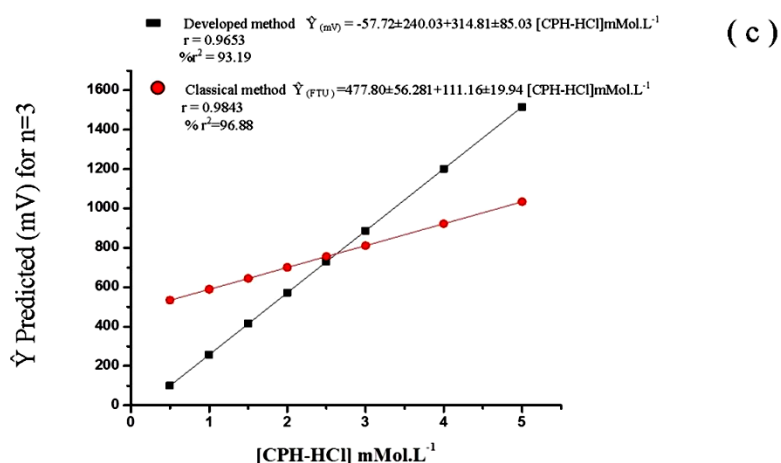


Fig.10 : Calibration graph for the variation of CPH-HCl concentration on:

(A) :Energy transducer response expressed as an average peak heights (mV) using Ayah 6SX1-T-2D Solar-CFI Analyser

(B) : FTU using HANNA instrument

(C): Y predicated ($\hat{Y}_i$) using two method (Ayah 6SX1-T-2D Solar-CFI Analyser & classical method (HANNA instrument))

Figure 10-C shows that the slope value using developed method is 314.18 that larger than the slope value using classical method (111.16) ; this indicate the developed method have a high sensitivity than classical method .

Final conclusion can be drawn that there is significant difference between two method, with the advantage having sensitivity and more linearity. A Choice is left for the analyst to choose between high sensitivity and linearity.

Assessment of the use of Ayah 6SX1-T-2D solar cell CFI Analyser for the determination of CPH-HCl in the pharmaceutical preparation using CPH-HCl-[Fe(CN)₆]³⁻

Three different companies of pharmaceutical preparations (Periahist – Iraq , Citadine – India , Nebore – Indonesia) . Continuous flow injection analysis using homemade Ayah 6SX1-T-2D-Solar cell CFI Analyser with six snow white light emitting diode as a source for measuring turbidity via reflecting of incident light . A series of solution were prepared of each pharmaceutical drug (5mmol.L⁻¹) (87.725mg , C₂₁H₂₂ClN.1.5H₂O, M.Wt:350.9 g.mol⁻¹,SDI of active ingredient in 50 mL) by transferring 6 ml to each five volumetric flask (10 mL) , followed by the addition of gradual volumes of standard CPH-HCl (0, 0.1, 0.2, 0.3, 0.4) mL of 0.05 mol.L⁻¹ to obtain (0, 0.5, 1, 1.5 , 2) mmol.L⁻¹ when use Ayah 6SX1-T-2D Solar cell –CFI Analyser , while transferring 3 ml to each five volumetric flask (25 mL) , followed by the addition of gradual volumes of standard CPH-HCl (0, 0.2, 0.4, 0.6, 0.8) mL of 0.05 mol.L⁻¹ to obtain (0,0.4,0.8,1.2,1.6) mmol.L⁻¹ for the use of HANNA instrument (classical method) . Flask no.1 is the sample .The measurements were conducted by both methods . Results were mathematically treated for the standard addition method .The results were tabulated in Table.12-A ,at confidence level 95% ; showing practically concentration of CPH-HCl in each pharmaceutical drug using two methods of analysis . Table.12-B was shown a practical content of active ingredient at 95% confidence level & efficiency of determination in addition to paired t-test which shows a comparison at two difference paths:

First: Individual t-test

A comparison between developed method using Ayah 6SX1-T-2D-Solar cell CFIA with quoted value . The obtained values suggest that there is no significant difference between the quoted value and calculated t-value as shown in Table .12-B column 8 .

Second:

Paired t-test was used in order to compare between developed method using Ayah 6SX1-T-2D-Solar cell CFIA with classical method as shown in Table. 12-B column 11 , the obtained result indicating clearly there was no significant different between two method at 95% confidence level ,since the calculation t-value less than $t_{tab}(4.303)$ for the determination of CPH-HCl in pharmaceutical preparation . Also F-test was used .

F-test is defined the ratio of variances of the two measurements, when the F-test is used , the larger variance is always placed in the numerator of the formula . The reason is that the F-test value is always greater than or equal to 1 .

F-test is provided a simple method for comparing the precision of two sets of measurements using the following equation

$F = S_1^2/S_2^2$ or S_2^2/S_1^2 ($F > 1$) , where , S_1^2 & S_2^2 is the variation

$$S_1^2 = \sum (x_i - \bar{X})_1^2 / n_1 - 1$$

$$\text{And } S_2^2 = \sum (x_i - \bar{X})_2^2 / n_2 - 1$$

($n_1 - 1$) and ($n_2 - 1$) = number of degrees of freedom for Ayah 6SXI-T-2D Solar cell CFI Analyser and classical method (measurement turbidity by HANNA instrument) respectively .

A hypothesis can be estimated as follow:

Null hypothesis

$H_0 : S_1^2 = S_2^2$ (no difference for any method used)

Against :

Alternative hypothesis

$H_1 : S_1^2 \neq S_2^2$

Form the results (Table 12-B – column 12) and the F-test value :

$$\begin{aligned} F &= S_2^2/S_1^2 \\ &= (0.1405)^2 / (0.036)^2 \\ &= 15.07 \end{aligned}$$

Since $\alpha = 0.05$ and this is a two –tailed test . The critical value for $F_{\alpha/2}^{v_1, v_2}$ (degree of freedom for Ayah 6SX1 –T-2D Solar cell CFIA : $v_1, n_1 - 1 \rightarrow 3 - 1 = 2$ & degree of freedom for classical method : $v_2, n_2 - 1 \rightarrow 3 - 1 = 2$) so $F_2^2 = 39$.

Since calculation F_{value} of $15.07 < F_{\alpha/2, 2, 2}$ (39) at 95% confidence level . Therefore; H_0 is accepted against H_1 , indicating that there is no significant difference between the performance of the two methods in terms precision (F-test) .

Table. 12-A: Standard addition results for the determination of CPH-HCl in three pharmaceutical preparation

No. of sample	Commercial name, Company Content Country	Sample weight equivalent to 87.725 mg (5 mMol.L ⁻¹) of the active ingredient (g)	Ayah 6SX1-T- 2D Solar cell CFIA							
			HANNA instrument (classical method turbidity measurement)							
			[CPH-HCl] mMol.L ⁻¹					Equation of standard addition at 95% for n-2 $\hat{Y}_{i(mV)} = a \pm s_a t + b \pm s_b t [x]$ $\hat{Y}_{i(FTU)} = a \pm s_a t + b \pm s_b t [x]$	r r ² r ² %	Practical concentration mMol.L ⁻¹ in *10,*25 ml
			0	0.5	1	1.5	2			
			0	0.4	0.8	1.2	1.6			
1	Periahist IPI 4 mg Iraq	2.772	800	900	1100	1180	1340	792.00±71.999+272.00± 58.787[x]	0.9932 0.9863 98.63	2.912 4.853
			240	500	650	945	1000	274.00±159.466+491.25±162.753[x]	0.9841 0.9685 96.85	0.558 4.650
2	Citadine Medico 4 mg India	5.256	630	745	848	870	1100	625.60±129.306+213.0±105.578 [x]	0.9655 0.9321 93.21	2.934 4.890
			147	284	423	520	620	162.40±46.645+295.50±47.606[x]	0.9962 0.9924 99.24	0.549 4.576
3	Nebor Dankos 4mg Indonesia	5.711	790	920	1060	1180	1340	786.00±25.456+272.00±20.785[x]	0.9991 0.9983 99.83	2.890 4.816
			290	394	726	897	986	279.60±176.276+473.75±179.910[x]	0.9793 0.9590 95.90	0.590 4.918

$\hat{Y}$ = Estimated response in mV for Ayah 6SX1-T-2D Solar cell CFIA or FTU using HANNA instrument, [x] = [CPH-HCl] mMol.L⁻¹ , r = Correlation coefficient , r²%= Linearity percentage , r² = Correlation of determination

* = 10 ml when use developed method & 25 ml when use classical method

Table. 12-B: Summary of results for paired t-test, practical content and efficiency for determination of CPH-HCl in three samples of pharmaceutical preparation using CPH-HCl-[Fe(CN)₆]³⁻ system .

Sample no.	Confidence interval for the average weight $\bar{W} \pm 1.96 \sigma_{n-1} / \sqrt{n}$ at 95% (g)	Sample weight equivalent to 87.725 mg (5mMol.L ⁻¹) of the active ingredient (g)	Theoretical content of the active ingredient at 95% (mg) $\mu \pm 1.96 \sigma_{n-1} / \sqrt{n}$	Practical concentration (mMol.L ⁻¹)and what is equivalent of active ingredient (mg)	Practical content $W_i \pm 4.303\sigma_{n-1}/\sqrt{n}$ (mg) for (n=3) ,at 95%	Efficiency of determination (Rec. %)	Paired t-test				F-test
				Ayah 6SX1-T- 2D Solar cell CFIA	HANNA instrument (classical method, turbidity measurement)	Individual comparison $(\bar{X} - \mu) \sqrt{n}/\sigma_{n-1}$ Ayah 6SX1-T- 2D Solar cell-CFI Analyser with Quoted value $t_{0.05/2, 2}=4.303$	Comparison between two method				
							Xd	$\bar{X}_d$ (σ_{n-1})	$t_{cal} = \bar{X}_d \sqrt{n} / \sigma_{n-1}$ at 95 %		
1	0.126 ± 0.002	2.772	4±0.077	4.853 85.145	3.870±2.43	96.75	-0.230 <<4.303	0.161	0.111 (0.172)	1.114 < 4.303	$(\sigma_{n-1})_1 = 0.036$ $S_1^2 = 0.00131$ $v_1=2$ $n_1=3$ $(\sigma_{n-1})_2=0.1405$ $S_2^2 = 0.0197$ $v_2=2$ $n_2= 3$ $F_{cal} < F_{tab}$ 15.07< 39
				4.650 81.590	3.709±3.06	92.72					
2	0.240±0.002	5.256	4±0.026	4.890 85.803	3.918±2.61	97.95	-0.135 <<4.303	0.252			
				4.576 80.291	3.666±2.68	91.66					
3	0.260±0.001	5.711	4±0.015	4.816 84.500	3.847±2.36	96.17	-0.279 <<4.303	-0.081			
				4.918 86.290	3.928±2.36	98.21					

Xd: Difference between two method , $\bar{X}_d$: difference mean , σ_{n-1} : Difference standard deviation , n= no. of sample =3

v_1, v_2 : Degree of freedom (dF) for two method (Ayah 6SX1-T-2D Solar cell CFIA & classical method (turbidity measurement))

n_1, n_2 : No. of samples , S_1^2 & S_2^2 : Variation for (6SX1-T-2D Solar cell CFIA & classical method (turbidity measurement))

$F_{0.05/2, 2, 2} = 39$.

Conclusion:

The proposed method for the determination of CPH-HCl based on the formation of yellow precipitate for ion-pair between the drug and $[\text{Fe}(\text{CN})_6]^{3-}$ in aqueous medium and measured the turbidity via the use of Ayah 6SX1-T-2D Solar cell CFIA . The method is a simple, sensitive , does not require reaction coil , expensive chemicals and without involve any specific sample treatment. In addition to easy and cheaper carry out. This method used for determination of CPH-HCl in nano gram for 100 μL sample volume in pure and pharmaceutical preparation.

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.

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