A new mode for an on-line determination of Ciprofloxacin in pure and pharmaceutical preparation using CFIA with homemade Ayah $3S_{BGR}$x3- 3D solar cell microphtometer analyzer.

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Abstract:

A new, simple, accurate, fast and sensitive spectrophotometric method has been developed for the analysis of ciprofloxacin in pure and pharmaceutical formulations by continuous flow injection analysis. The method was based on the oxidation of the drug with Ce(IV)sulfate in acidic medium to formed a pinkish-red color species which determined using homemade Ayah $3S_{BGR}$ x3-3D solar cell flow injection photometer. Optimum conditions were obtained using a high intensity green light emitted diode as a source. The linear dynamic range for the instrument response versus ciprofloxacin concentration was 0.05-12 mmol.L^{-1} while the L.O.D was of 0.300 µg / sample. The correlation coefficient (r) was 0.9912 while percentage linearity (r^2%) was 98.25%. RSD% for the repeatability (n=8) was lower than 0.6% for the determination of ciprofloxacin, at concentration of 12 mmol.L^{-1}. The method was applied successfully for the determination of ciprofloxacin in pharmaceutical preparation. A comparison was made between the newly developed method and the classical method (UV-Vis spectrophotometry) at wave length 551nm of analysis using the standard additions method via the use of paired t-test. It showed that there was no significant difference between the quoted value of each individual company with calculated t-value at 95% confidence interval from developed method, in addition to comparison between two methods and calculate t-value, it was noticed that there was no significant difference between two methods at 95% .

نمط جديد للتقدير الأني للسايبروفلوكساسين في المستحضرات الصيدلانية والنقية بأستخدام التحليل بالحقن الجرياني المستمر عن طريق استخدام محلل طيفي مايكروي مصنع محليا Ayah $3S_{BGR}$ x3-3D solar cell.

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الكلمات المفتاحية : سايبروفلوكساسين, التحليل بالحقن الجرياني, التحليل الطيفي.

الخلاصة:

طورت طريقة طيفية جديدة, بسيطة, سريعة, مظبوطة, وحساسة لتحليل السايبروفلوكساسين في صيغته النقية والمستحضرات الصيدلانية بأستخدام التحليل بالحقن الجرياني المستمر. استندت الطريقة على اكسدة العقار بكبريتات السيريوم (VI) في الوسط الحامضي لتكوين اصناف ملونة وردي- محمر والتي قدرت بأستخدام مطياف الحقن الجرياني مع Ayah $3S_{BGR}$ x3-3D solar cell المصنع محليا. تم دراسة الظروف الفضلى التي تم التوصل اليها بأستخدام ثنائي الوصلة باعث للضوء الاخضر وبشدة عالية كمصدر للتشعيع. كان المدى الخطي الفعال لعلاقة تغير الاستجابة الألية مع تركيز السايبروفلوكساسين 0.05 - 12 مللي مول.$لتر^{-1}$ بينما حدود الكشف 0.300 مكغم / انموذج. وان معامل الارتباط (r) 0.9912 بينما نسبة الخطية r^2 % = 98.25 % . الانحراف القياسي النسبي المؤي (RSD%) للتكرارية اقل من 0.6 % لمحلول السايبروفلوكساسين بتركيز 12 مللي مول .$لتر^{-1}$ (8=n) طبقت الطريقة بنجاح لتقدير السايبروفلوكساسين في المستحضرات الصيدلانية .

اجريت مقارنة بين الطريقة المطورة والطريقة التقليدية (مطيافية UV-Vis) عند طول موجي 551 نانو متر للتحليل باستخدام منحني الاضافات القياس من خلال استخدام اختبار t- المزدوج. تبين انه لايوجد فرق جوهري بين القيمة الرسمية وقيمة t- المحسوبة من الطريقة المطورة عند فاصل ثقة 95% بالاضافة الى اجراء مقارنة بين الطريقتين وحساب قيمة t- ولوحظ انه لايوجد فرق جوهري بين الطريقتين عند مستوى قناعة 95% .

Introduction:

Ciprofloxacin (Cip, figure 1) is chemically 1-cyclopropyl-6-fluoro-4-oxo-7-(piperazin-1-yl)-quinoline-3-carboxylic acid, Ciprofloxacin hydrochloride is the monohydrochloride monohydrate salt of ciprofloxacin [1,2]. And belongs to the group of synthetic fluoroquinolone antibiotics with broad antimicrobial activity [3]. Cip is a quinolone antibacterial agent with fluorine at position 6 of naphthyridine ring [4]. Cip is a second-generation fluoroquinolone antibiotic [5,6] . Cip is structurally related to nalidixic acid. It is believed that the mode of action of this family of drugs is through binding DNA-gyrase enzyme[7] . It is also reported that there is a direct correlation of fluoroquinolone bonding with inhibition of DNA-gyrase enzyme activity and induction of DNA breakage. Because of this special mechanism of action, fluoroquinolones are considered to be the most effective gram-positive-gram-negative pathogens to combat infections caused by microorganisms that are resistant to other microbials, such as tetracycline [8,9] . Its spectrum of activity includes most strains of bacterial pathogens responsible for respiratory, urinary tract, gastrointestinal, and abdominal infections, including Gram-negative (Escherichia coli, Haemophilus influenzae, Klebsiella pneumoniae, Legionella pneumophila, Moraxella catarrhalis, Proteus mirabilis, and Pseudomonas aeruginosa), and Gram-positive (methicillin-sensitive but not methicillin-resistant Staphylococcus aureus, Streptococcus pneumoniae, Staphylococcus epidermidis,Enterococcus faecalis, and Streptococcus pyogenes) bacterial pathogens. Cip and other fluoroquinolones are valued for this broad spectrum of activity, excellent tissue penetration, and for their availability in both oral and intravenous formulations [10]. Cip is the most widely used of the second generation quinolone antibiotics that came into clinical use in the late 1980s and early 1990s [11]. Cip was first patented in 1983 by Bayer A.G. and subsequently approved by the US Food and Drug Administration (FDA) in 1987. Cip has 12 FDA-approved human uses and other veterinary uses [12].

Many methods have been reported for determination of Cip, High performance liquid chromatography (HPLC) [13-17]. Spectrophotometry [18- 22]. Capillary electrophoresis [23, 24]. Chemiluminescence[25]. Turbidity 0-180° - FIA [26].

The proposed method based on the oxidation of Cip by Ce (IV) sulfate in acidic medium. The oxidation product yields a pinkish red color measured by Ayah $3S_{BGR}x3$-3D solar cell CFIA microphotometer (homemade) [27] at 525 nm. The procedure is simple, rapid and is proposed for the control analysis of the drug in pure and pharmaceutical preparation as an alternative analytical procedure.

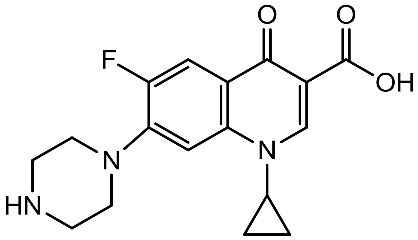


Figure 1: Chemical structure of Cip.

Experimental:

Chemicals:

All chemicals were used of analytical- grade reagent while distilled water was used to prepare the solutions. A standard solution 0.05 M of Cip ($C_{17}H_{18}FN_3O_3.HCl.H_2O$, M.Wt. 385.82 g.mol^{-1}, SDI) was prepared by dissolving 4.8228 g in 250 ml of distilled water. A stock solution of Ce(IV) sulfate 0.1M ($Ce(SO_4)_2$, M.Wt 332.298 g.mol^{-1} , Hopkin & Williams LTD) was prepared by dissolving 16.615 g in 500 ml of sulphuric acid 1M, (H_2SO_4, M.Wt 98g.mol^{-1}, 18 M, sp.g 1.84g.ml^{-1} , percentage 96%, BDH,) was prepared by diluting 27.7ml of sulphuric acid in distilled water to final volume 500ml by using volumetric flask 500 mL. Hydrochloric acid solution1M (35%, 1.19 g.ml^{-1}, BDH) were prepared by pipetting 21 mL of concentrated hydrochloric acid and completed of the volume with distilled water in 250 mL volumetric flask. Nitric acid solution 1M (70%, 1.42 g.ml^{-1}, BDH) was prepared by pipetting 16 mL of concentrated nitric acid and completed the volume with distilled water into 250 mL-volumetric flask. Phosphoric acid solution 1M (85%, 1.69 g.ml^{-1},BDH) was prepared by pipetting 17 mL of concentrated phosphoric acid and complete the volume with distilled water to 250 ml-volumetric flask. All acids were standardized with Na_2CO_3 solution.

Sample preparation:

Thirteen tablets were weighted crushed and grinded. Tablets containing 500 mg of Cip were weighted, 3.010 g of ciprova- India, 2.9884 g of cipropharm- Jordan, and 2.97553 g of ciproxene-Syria, equivalent to 1.9291 g of active ingredient to obtain 50 mmol.L^{-1}. The powder was dissolved in little distilled water followed by filtration to get rid of dissolved materials, then completed the volume to 100 ml with distilled water.

Apparatus and manifold:

The flow system used for the determination of Cip, shown schematically in figure 2, peristaltic pump four channels variable speed (Ismatec, Switzerland). Valve 6 – port medium pressure injection valve (I D E X corporation , USA) with sample loop (1 mm i.d. Teflon ,variable length) . The instrument response was measured by Ayah 3S_{BGR} x 3 – 3 D solar cell continuous flow injection analysis microphotometer (homemade) [27] by using super bright blue470 nm , green 525nm and red 635 nm emitted diode (LED) as a source , three solar cell as a detector. The output signals were recorded by potentiometric recorder (Siemens, Germany,1- 500 Volt, 1-500 mV). Peak height was measured for each signal. UV-Vis Spectrophotometer digital double beam type (UV-Vis spectrophotometer: UV-1800, shimadzu, and spectronic 20D +, Japan) were also used to scan the spectrum of colored species using 1cm glass cell.

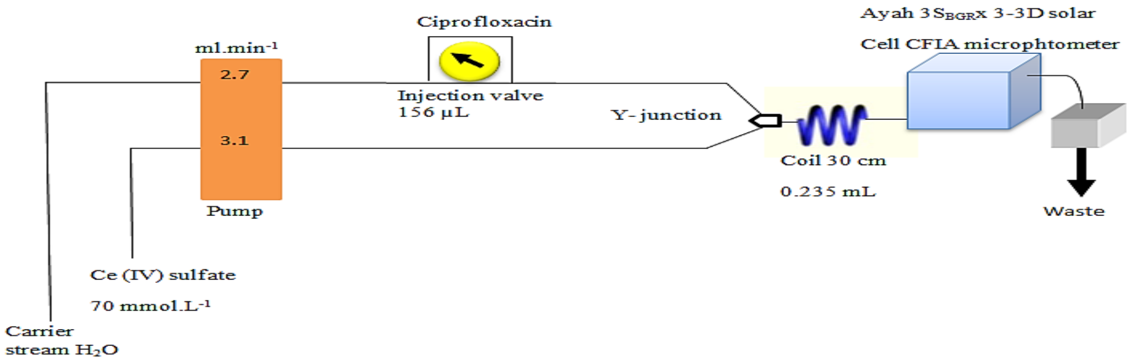
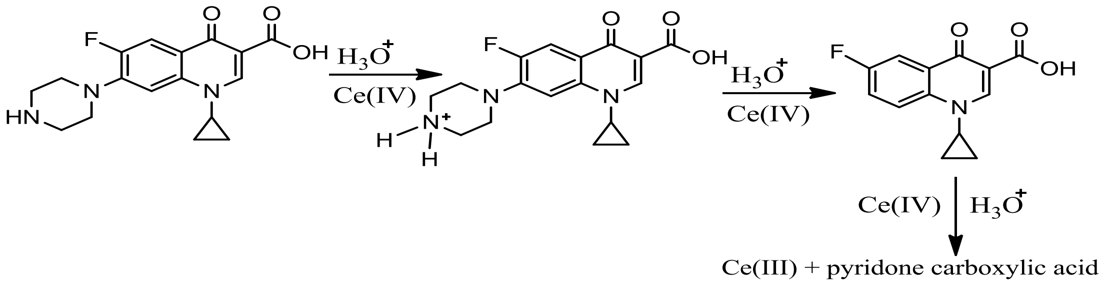


Figure 2: schematic diagram of Continuous Flow Injection Analysis system with Ayah 3S_{BGR} x 3 - 3D solar cell CFIA microphotometer.

Methodology:

The whole reaction manifold system for Cip determination via direct oxidation of Cip by Ce(IV) sulfate in acidic medium to form colored species was shown in figure 2 . The manifold system is composed from two lines: The first line supplied distilled water at 2.7 ml.min^{-1} which leads to the injection valve for carrying Cip sample with 156 µ L sample volume (loop length 19.82 cm , with 1mm I.D.) , the second line supplied Ce(IV) sulfate 70 mmol.L^{-1} in acidic medium (500 mmol.L^{-1} of H_2SO_4) at 3.1 ml.min^{-1} . Both of lines meet at junction (Y- junction) with an outlet for reactants product from pinkish – red colored species which passes through mixing coil (30cm, 0.235 ml) and then passed through Ayah 3S_{BGR} x3- 3D solar cell CFIA microphotometer. The variation of response was monitored using green light emitted diode LED (525 nm) throughout the reaction to obtain transducer energy response in mV versus time. Each solution was assayed triplicate. A proposed oxidation mechanism of Cip by Ce(IV)sulfate in acidic medium is presented in scheme(1) [28,29].



Schematic (1): proposed mechanism of reaction between Cip and Ce(IV) sulfate in acidic medium.

Results and discussion:

Scanning of spectrum for Cip – Ce(IV) sulfate – H_3O^+ system :

A scanning between 200-1100 nm was carried out to obtained λ_{max} for colored product (pinkish – red) by oxidation of Cip (2 $mmol.L^{-1}$) with Ce(IV)sulfate (10 $mmol.L^{-1}$) in acidic medium (H_2SO_4, 500 $mmol.L^{-1}$). It can be seen clearly that a maximum absorbance at 551 nm against reagent blank (Ce(IV) sulfate), as shown in figure 3-A. Second spectroscopic study for colored species (pinkish-red) was monitoring by the homemade Ayah $3S_{BGR}x3$-3D solar cell CFIA microphotometer at three different super bright light emitted diode (LED) including blue 470nm, green 525 nm, red 635 nm, selected conditions were used, two lines system, and 165 µL as a sample volume of Cip (10 $mmol.L^{-1}$) which injected into the carrier stream (H_2O), while the Ce(IV) sulfate 50 $mmol.L^{-1}$ (second line) meet with Cip at Y- junction point in later stage of reaction. A maximum response measured in mV (expressed as transducer energy response) which obtained using high intensity super bright green light emitted diode (LED) as shown in Figure 3-B. Therefore, the green light emitted diode (LED) will be used as a best source in this work.

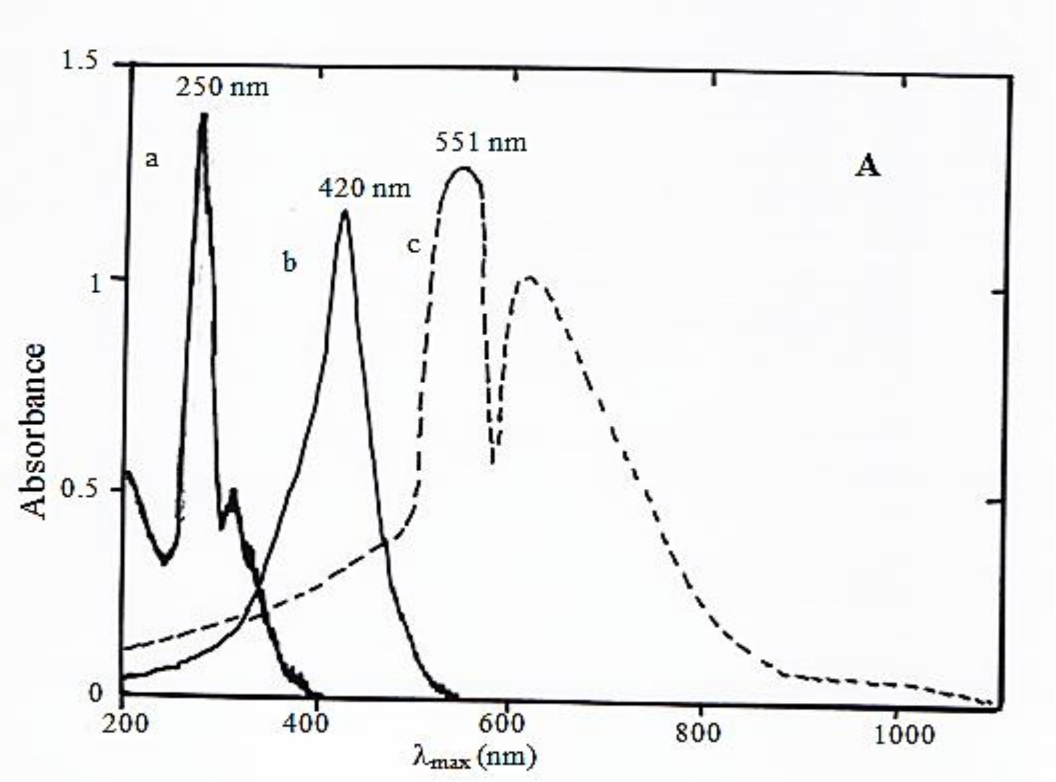


Figure 3-A: Absorbance spectra for: a: Cip, b: Ce(IV) sulfate, c: Cip-Ce(IV) sulfate-H_3O^+ system.

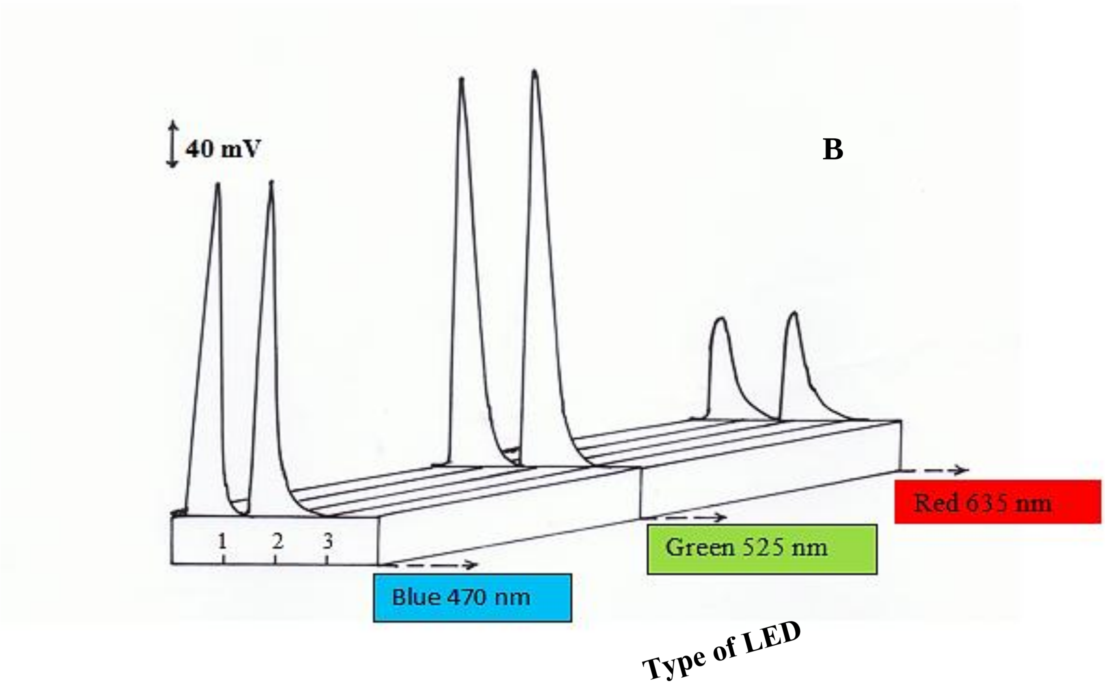


Figure 3-B: responses profile (mV) verses light emitted diode using Ayah 3S$_{BGR}$ x 3 –3D solar cell CFIA microphtometer for Cip (10 mmol.L^{-1}) – Ce(IV) sulfate (50 mmol.L^{-1}) – H_3O^+ system, with 165 µL as a sample volume, flow rate 2.1, and 2.4 ml.min^{-1}.

Optimum conditions for oxidation of Cip with Ce (IV) Sulfate in acidic medium.

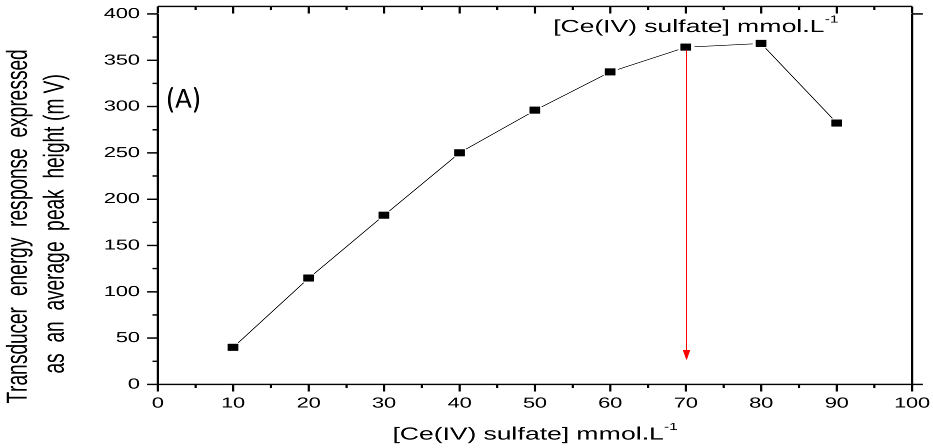
Chemical variables:

Effect of Ce(IV) sulfate concentration:

The effect of varying concentration of Ce(IV) sulfate Was examined, using a series of solutions ranged 10 – 90mmol.L^{-1} which diluted by 500 mmol.L^{-1} of H_2SO_4, at 10 mmol.L^{-1} of Cip and 165 µL as a sample volume were used. Flow rate was used at 2.1, and 2.4 ml.min^{-1} for the carrier stream, and Ce(IV) sulfate respectively. The obtained results were tabulated in Table. 1, which Summarizes the average of three successive readings with relative standard deviation and confidence interval of the average response at 95% confidence ($\propto = 0.05$). Figure 4 shows the plot and response profile of the results as it obtained from Ayah 3S$_{BGR}$ x3 -3D solar cell CFIA microphotometer. It was noticed that an increase in the response of the colored species with increasing Ce (IV) Sulfate concentration up to 70 mmol.L^{-1} more than 70 mmol.L^{-1} led to broadening in high peak maxima and increase the peak base width (Δt_b), might be due to increase the intensity of colored species which work as internal filter in the front of detector that prevent remaining light after absorption process from passes to the solar cell, in addition to form a precipitate particles causing the restriction of flow. Therefore, 70 mmol.L^{-1} of Ce(IV) sulfate was chosen as optimum concentration in next studies to give a maxima peak height and sharp.

Table. 1: variation of Ce (IV) sulfate concentration on the transducer energy responseof Cip – Ce(IV)-H_3o^+ system .

[Ce(IV)sulfate] mmol.L^{-1}	Transducer energy response expressed as peak height n=3 , Y_i (mV)	Average transducer energy response expressed as peak height n=3, $\square_i$ (mV)	RSD%	Confidence interval of the average response $\square_i \pm t_{0.05/2,\, n-1}\ \sigma_{n-1}/\ \sqrt{n}$
10	40 , 40 , 40	40.00	0.0	40 ± 0.0
20	116 , 116 , 112	114.67	2.0	114.67 ± 5.71
30	184 , 184 , 180	182.67	1.25	182.67 ± 5.71
40	252 , 248 , 250	250.00	0.80	250 ± 4.97
50	300 , 292 , 296	296.00	1.35	296 ± 9.93
60	340 , 336 , 336	337.33	0.68	337.33 ± 5.71
70	364 , 364 , 364	364.00	0.0	364 ± 0.0
80	368 , 368 , 368	368.00	0.0	368 ± 0.0
90	280 , 284 , 282	282.00	0.70	282 ± 4.97



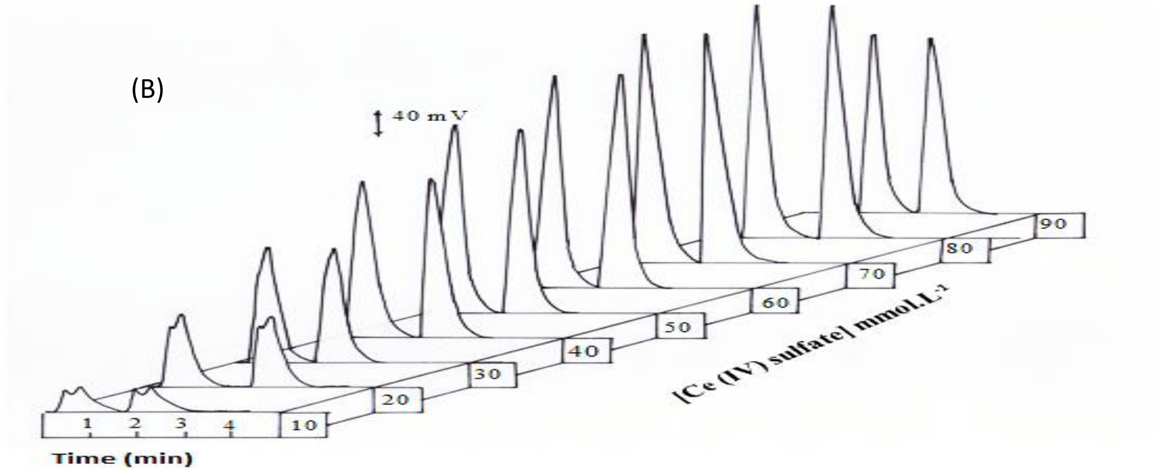


Figure 4 :variation of [Ce(IV) sulfate] on: (A) transducer energy response (mV), (B) response profile using Ayah $3S_{BGR}X$ 3-3D solar cell CFIA microphotometer.using 10 mmol.L^{-1} of Cip (165µL), flow rate 2.1, and 2.4 ml.min^{-1}.

Effect of acidic medium:

The reaction of Cip with Ce(IV) sulfate can be conducted in different acidic medium (H_2SO_4, HCl, HNO_3, H_3PO_4, at 50 mmol.L^{-1}) in addition to H_2O as a carrier stream at 2.1 ml.min^{-1}. The optimum concentration of Ce(IV) sulfate 70 mmol.L^{-1} (in 500 mmol.L^{-1} of H_2SO_4) at 2.4 ml.min^{-1}, 10 mmol.L^{-1} of Cip with 165µL sample volume were used. Table .2 shows the obtained results, while figure 5 shows the plot, it can be seen there is no significant different among acids except H_3PO_4

was given a higher response but it was avoided because of form a precipitate of Ceric phosphate which causes the restriction of flow and closing the manifold of the system and leading to distortion the response and increase base width. The distilled water was chosen as optimum carrier stream, because of its availability, non-toxicity, and cheap as well as being a suitable medium for the sensitivity and to obtain the best response height.

Table .2 : variation PH of the carrier on the transducer energy response of Cip – Ce(IV)-H_3o^+ system.

Acid type	Transducer energy response expressed as peak height n=3 , Y_i (mV)	Average transducer energy response expressed as peak height n=3, $□_i$ (mV)	RSD%	Confidence interval of the average response $□_i \pm t_{0.05/2,\ n-1}\ \sigma_{n-1}/\ \sqrt{n}$
H_2O	418, 416, 418	417.33	0.28	417.33 ± 2.85
H_2SO_4	412, 410, 408	410.00	0.48	410 ± 4.96
HCl	400, 400, 402	400.67	0.28	400.67 ± 2.85
HNO_3	404, 404, 400	402.67	0.57	402.67 ± 5.71
H_3PO_4	592, 588, 580	590.00	0.33	590 ± 4.96

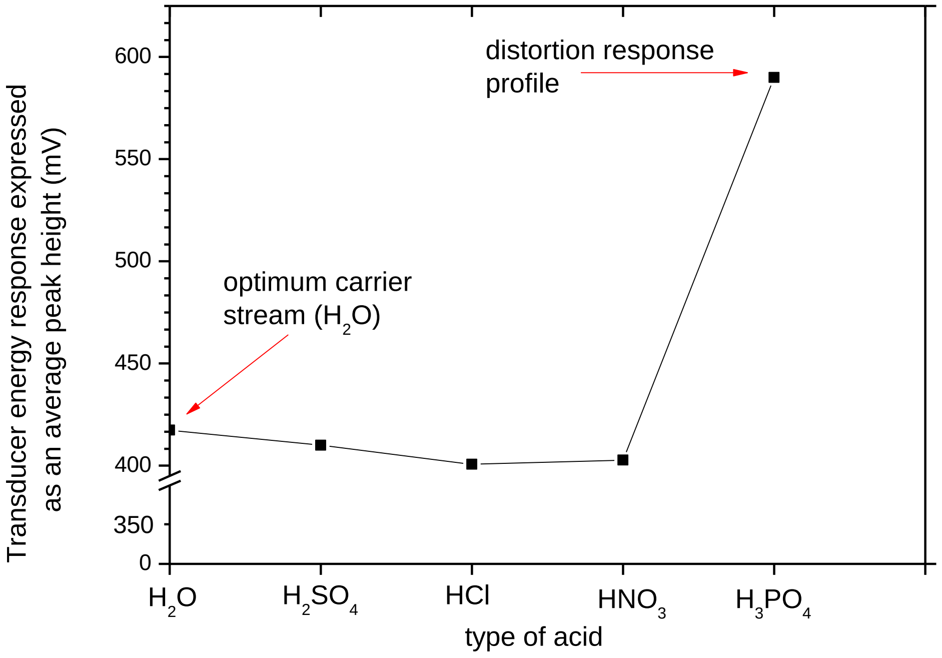


Figure 5: variation type of acidic medium as a carrier stream on the transducer energy response (mV), for determination of Cip.

Physical variables:

Flow rate:

The flow rate ranged 0.4-2.7, and 0.4-3.1 $ml.min^{-1}$ for the carrier stream and the reagent (Ce(IV) sulfate) respectively of the manifold system to determination of Cip was investigated to conducted the optimize of preferred flow rate. The optimum concentration 70 $mmol.L^{-1}$ of Ce(IV) sulfate was used, and using 10 $mmol.L^{-1}$ of Cip with 165 µL as a sample volume, while distilled water was used as a carrier stream. The obtained results were tabulated in Table .3, while figure 6 shows the plot. It was noticed that at low flow rate there were an increase in peak base width, with decrease peak height, and broadening at the peak maxima, which might be attributed to the dispersion and dilution leading to an irregular response profile. But at high flow rate influence (up to pump speed 40) led to an increase in peak height, decrease the peak base width, and decrease time that required for arrive the colored species to the measuring flow cell. As compromise between sensitivity, peak shape, complete the reaction, and consumption of the chemicals, 2.7, and 3.1 ml.min^{-1} will be used as optimum flow rate for the carrier stream and Ce(IV) sulfate respectively.

Table. 3: variation of flow rate (ml.min^{-1}) on the transducer energy response (mV).

Pump speed indication approximate	Flow rate (ml.min^{-1})		Average transducer energy response expressed as peak height n=3, □$_i$ (mV)	RSD%	Confidence interval of the average response □$_i$ $\pm t_{0.05/2,\, n-1}\, \sigma_{n-1}/\sqrt{n}$	Δt_b sec	t sec
	Carrier stream (H_2O)	Ce(IV) sulfate line					
5	0.4	0.4	140.00	0.0	140 ± 0.0	462	66.0
10	0.7	0.7	152.00	1.31	152 ± 4.96	228	42.0
15	1.1	1.2	295.33	0.77	295.33 ± 5.71	162	27.0
20	1.4	1.6	358.00	0.55	358 ± 4.96	138	21.0
25	1.8	2.0	380.00	1.05	380 ± 9.93	108	18.0
30	2.1	2.4	396.00	0.0	396 ± 0.0	96	16.2
35	2.4	2.8	448.00	0.77	448 ± 5.99	84	15.0
40	2.7	3.1	468.67	0.24	468.67 ± 2.85	66	12.0

Δt_b (sec): peak base width

t (sec) : time for the departure of sample segment from injection valve reaching to the measuring flow cell .

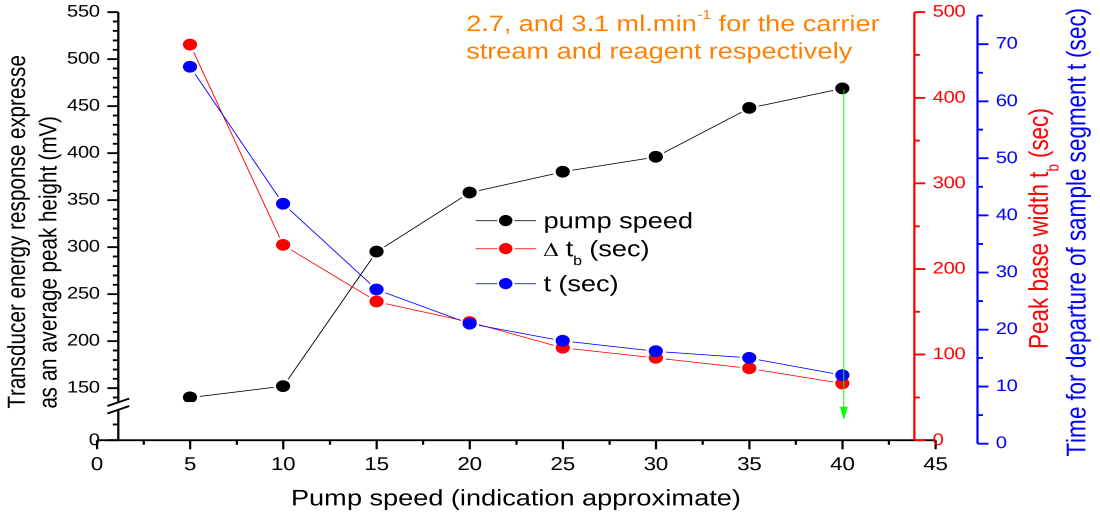


Figure 6: variation of flow rate on transducer energy response, peak base width (Δt_bsec), and time for the departure of sample segment from injection valve reaching to the measuring flow Cell (t sec).

Sample volume:

The study carried out using 2.7, and 3.1 ml.min^{-1} as optimum flow rate for the carrier stream and Ce(IV) sulfate (70 mmol.L^{-1} in acidic medium) respectively, with 10 mmol.L^{-1} of Cip. Variable sample volumes were used which equivalent to 110, 156, 165, 211, and 279 µL and successively used with open valve mode. The results were tabulated in Table. 4, which indicated that 156 µL was the optimum sample volume, which gave sharp and smooth response profile, in addition to economy and decrease in the analysis time. It was noticed that an increase in sample volume led to an increase in the height of response profile without effecting on the response profile up to the sample volume 156 µL. Above 156µL there were a broadening at the peak maxima and an increase in the base width (Δt_b) this is illustrated in figure 7 which shows these effects, and the kind of response profile.

Table. 4: variation of injected sample volume on transducer energy response.

Loop length (cm)	Sample volume (µL)	Average transducer energy response expressed as peak height n=3, □$_i$(mV)	RSD%	Confidence interval of the average response □$_i$ $\pm t_{0.05/2,\ n-1}\ \sigma_{n-1}/\sqrt{n}$	Δt_b Sec
14.00	110	338.67	0.33	338.67 ± 2.85	51
19.82	156	380.00	0.0	380 ± 0.0	54
21.00	165	372.00	0.53	372 ± 5.71	66
26.80	211	440.00	0.56	440 ± 4.96	70
35.60	297	392.67	0.29	392.67 ± 2.85	74

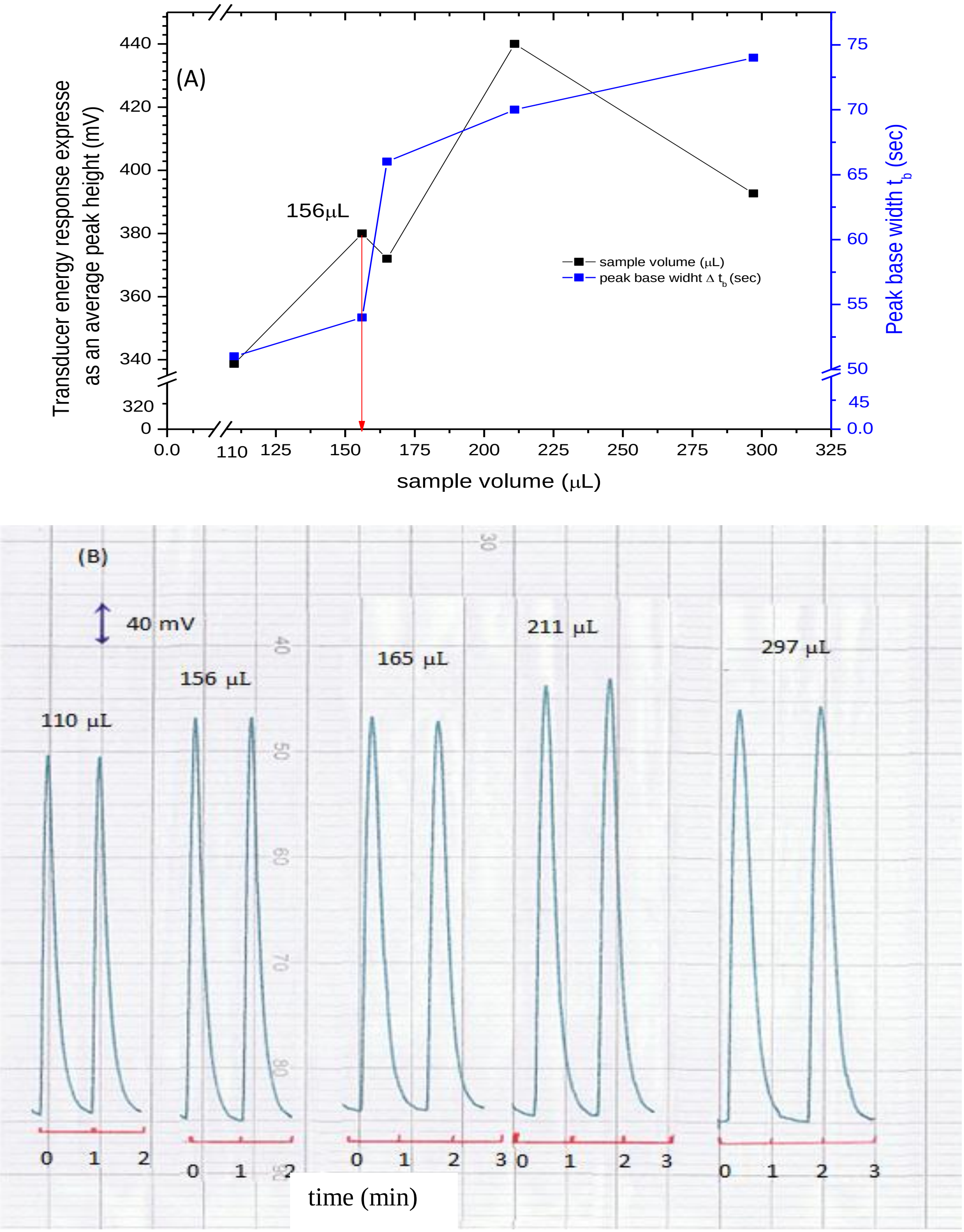


Figure 7: Variation of sample volume on: (A) transducer energy response, (B)response profile using Ayah 3S$_{BGR}$X 3-3D solar cell CFIA microphotometer for the determination of Cip.

Purge time:

Using different purge time for the sample segment i.e, the allowed time was 2-25 sec for the cip sample to passing through the injection valve (injection mode) followed by turning the injection valve to the load position. Optimum sample volume of cip (10 mmo.L^{-1}) 156 µL was used. Figure 8 shows the continuation of the increase in the height of response and Δt$_b$ with increase of injection time up to open valve mode, therefore open valve mode as purge time was chosen as optimum time to the complete purge of sample from injection valve which gave a better response profile. The obtained results were tabulated in Table .5.

Table. 5: Variation of purge time on the transducer energy response.

Purge time (Sec)	Average transducer energy response expressed as peak height n=3, □$_i$ (mV)	RSD%	Confidence interval of the average response □$_i$ ±$t_{0.05/2,\, n-1}\ \sigma_{n-1}/\sqrt{n}$	Δt$_b$ (sec)
2	231.33	1.79	231.33 ± 10.33	40
5	292.00	0.0	292 ± 0.0	42
7	304.00	0.0	304 ± 0.0	46
10	307.33	0.37	307.33 ± 2.85	48
15	309.33	0.74	309.33 ± 5.71	50
20	310.67	0.37	310.67 ± 2.85	52
25	336.00	0.0	336 ± 0.0	52
Open valve	376.00	0.0	376 ± 0.0	56

Δt$_b$ (sec):- peak base width.

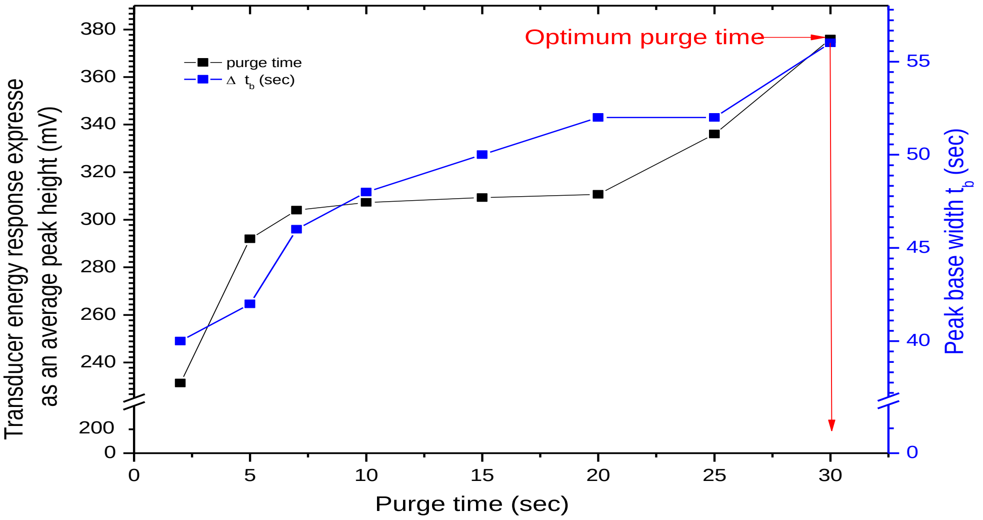


Figure 8: variation of purge time on the transducer energy response, and peak base width (Δt$_b$ sec).

Effect of reaction coil length:

The reaction coil length has a large role in the homogenization and completion of chemical reaction. The effect of reaction coil length on response profile was studied. Variable coil length 0 – 100 cm was used, this range of length comprises a volume of 0 – 0.785 mL which connected after Y-junction directly in flow system (Figure 2). Optimum concentration of Ce(IV) sulfate (70 mmol.L^{-1} in 500 mmol.L^{-1} of H_2SO_4) and 10 mmol.L^{-1} of Cip with sample volume 156 µL were used, while flow rate was 2.7, 3.1 ml.min^{-1} for the carrier stream and Ce(IV) sulfate respectively. Table. 6 shows all the obtained results of coil length effect on energy transducer energy response, peak base width (Δt_b), and Cip concentration after dilution, as shown in column 9. It can be seen that an increase in peak height with increase coil length, while increasing of coil length more than 30 cm gave broadening of the peak maxima, increase of the Δt_b, and increase arrival time of injected sample from injection valve to the measuring flow cell, which might probably attributed to the increase effect of dilution and dispersion on colored segment and continuous longer time duration of colored species in front of detector. Figure 9 shows the effect of coil length on transducer energy response and response profile, all that prove the best coil length for the completion of the oxidation of Cip by Ce(IV) sulfate in acidic medium is 30 cm (0.235 mL), while figure 2 shows the manifold system for determination of Cip using coil (30 cm) after Y – junction.

Table. 6: Variation of coil length on energy transducer response.

Coil length (cm)	Volume (ml) $r^2\pi h$, r = 0.5 mm	Average transducer energy response expressed as peak height n=3, $\square_i$ (mV)	RSD %	Confidence interval of the average response $\square_i \pm t_{0.05/2,\, n-1}\, \sigma_{n-1}/\sqrt{n}$	Δt_b (sec)	t (Sec)	V_{add} (ml)	[cip] mmol.L^{-1} after dilution
0.0	0.0	376.00	0.59	376 ± 5.16	66	72	7.14	0.22
30	0.235	408.00	0.0	408 ± 0.0	69	78	7.72	0.20
60	0.471	397.00	0.25	397 ± 2.48	72	82	8.11	0.19
100	0.785	384.00	0.0	384 ± 0.0	75	86	8.49	0.18

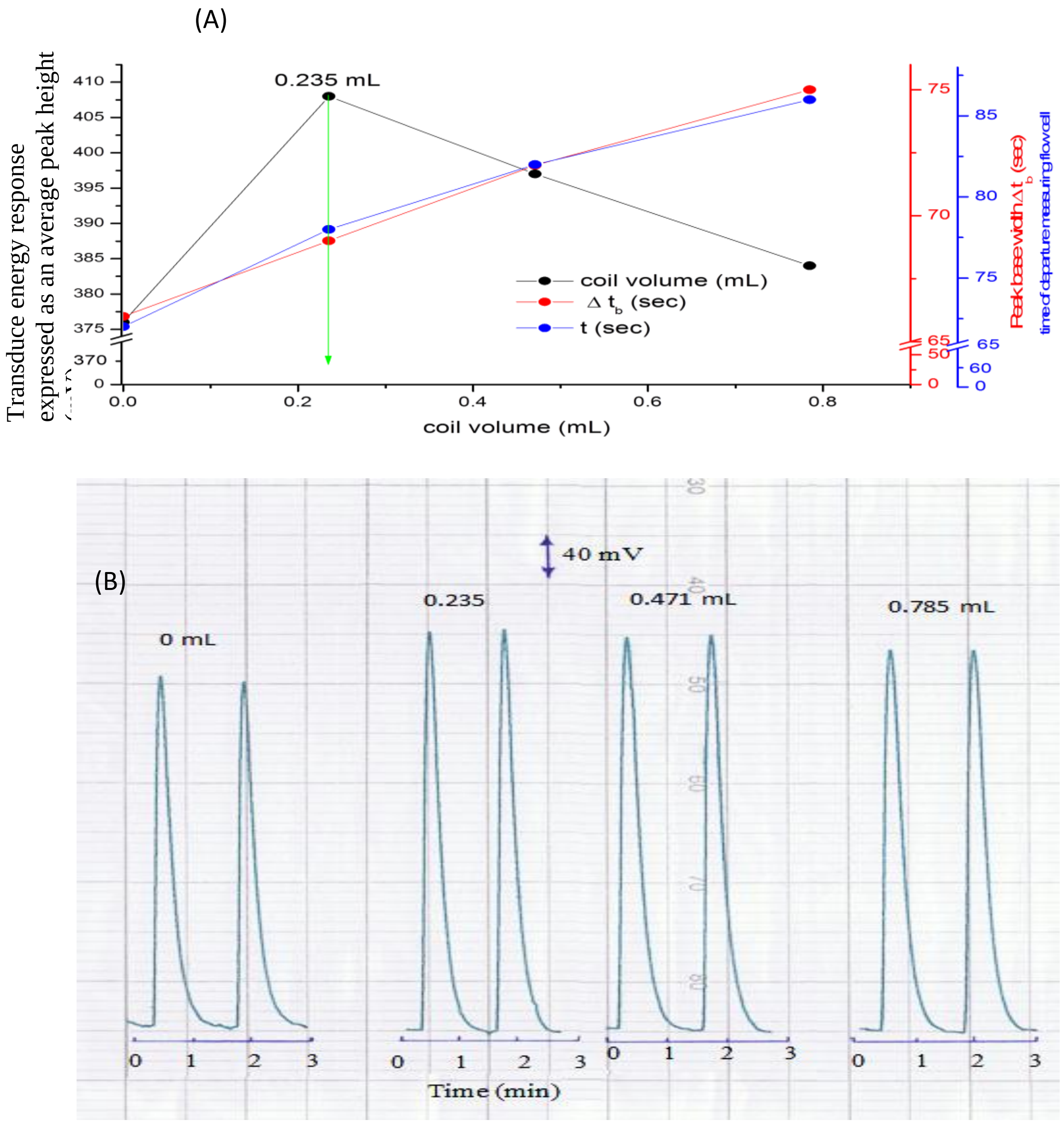


Figure 9: Variation of coil length on; A- energy transducer response, B- response Profile using Ayah $3S_{BGR}$ x 3-3D solar cell CFIA microphotometer determination of Cip 10 mmol.L^{-1} with 156 µL sample volume.

Incident light intensity

Variable intensity of light source was used 0.6 – 1.3 volt by variation of light intensity channel in Ayah $3S_{BGR}$ x 3- 3D solar cell CFIA microphotometer operation where read by AVO-meter. The optimum conditions were used; green light emitted diode (LED) 525 nm as an irradiation source, 70 mmol.L^{-1} of Ce(IV) sulfate, reaction coil (30 cm), flow rate was 2.7, and 3.1 ml.min^{-1} for the carrier stream and reagent line respectively, and sample volume was 156 µL of Cip (10 mmol.L^{-1}). The obtained results tabulated in Table. 7 which shows that an increase in the peak height with increase light intensity, therefore 1.3 volt was chosen as optimum voltage that can be supplied to give a better reproducible outcome as shown in figure 10; which explain the strong linear relation between the change of energy transducer output with light intensity.

Table . 7 : variation of incident light intensity on transducer energy response.

Intensity of Source (volt)	Average transducer energy response expressed as peak height n=3, $\square_i$ (mV)	RSD%	Confidence interval of the average response $\square_i \pm t_{0.05/2,\ n-1}\ \sigma_{n-1}/\sqrt{n}$
0.60	243.33	0.47	243.33 ± 2.85
0.70	280.00	0.0	280 ± 0.0
0.80	320.00	0.0	320 ± 0.0
0.90	358.00	0.0	358 ± 0.0
1.0	392.00	0.0	392 ± 0.0
1.10	426.00	0.47	426 ± 4.97
1.20	456.00	0.43	456 ± 4.7.0
1.30	478.33	0.43	478.33 ± 5.16

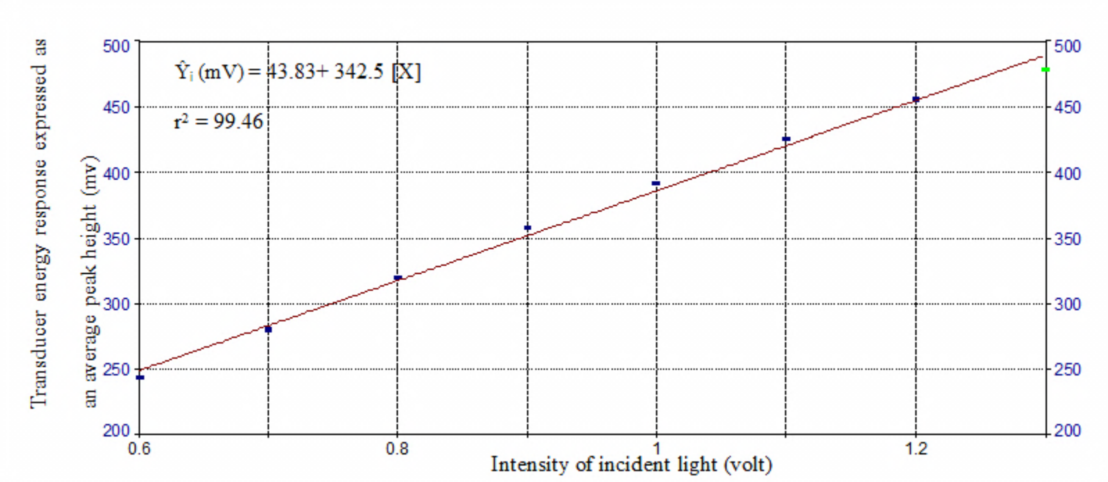


Figure 10: Variation of incident light intensity on transducer energy response for the Cip – Ce(IV)- H_3O^+ system.

[X] = intensity of incident light (volt).

Calibration graph:

At the established optimum conditions, a series of solution for cip (0.05 - 25 mmol.L^{-1}) were prepared. Each measurement was repeated three times. Transducer energy response of the average peak height (mV) was plotted against the concentration of cip. A straight –line graph (figure 11) from 0.05 – 12 mmol.L^{-1} of cip was obtained . Above 12 mmol.L^{-1} the value for correlation coefficient will decrease and deviate from linearity most probably due to the increase of the colored species in front of detector and due to the effect of inner filter of colored species present in solution, which results From the oxidation of cip by Ce(IV)sulfate in acidic medium which might be due to a decrease in transmitted light. The summary of results were tabulated in Table. 8.

Table. 8: summary of calibration graph results for the determination of Cip using Cip-Ce(IV) - H_3O^+ system [30,31]

Measured [cip] $Mmol.L^{-1}$	Linear dynamic range $mmol.L^{-1}$ n = 11	Ŷi (mV) = a ± ts_a +b ± ts_b [cip] $mmol.L^{-1}$ at confidence level 95% , n – 2	r r^2 %	t tab	$t_{cal} = /r/ \frac{\sqrt{n-2}}{\sqrt{1-r2}}$
0.05-25	0.05-12	17.08 ± 25.24 + 46.21 ± 4.63	0.9912 98.25	2.262 << 22.53	

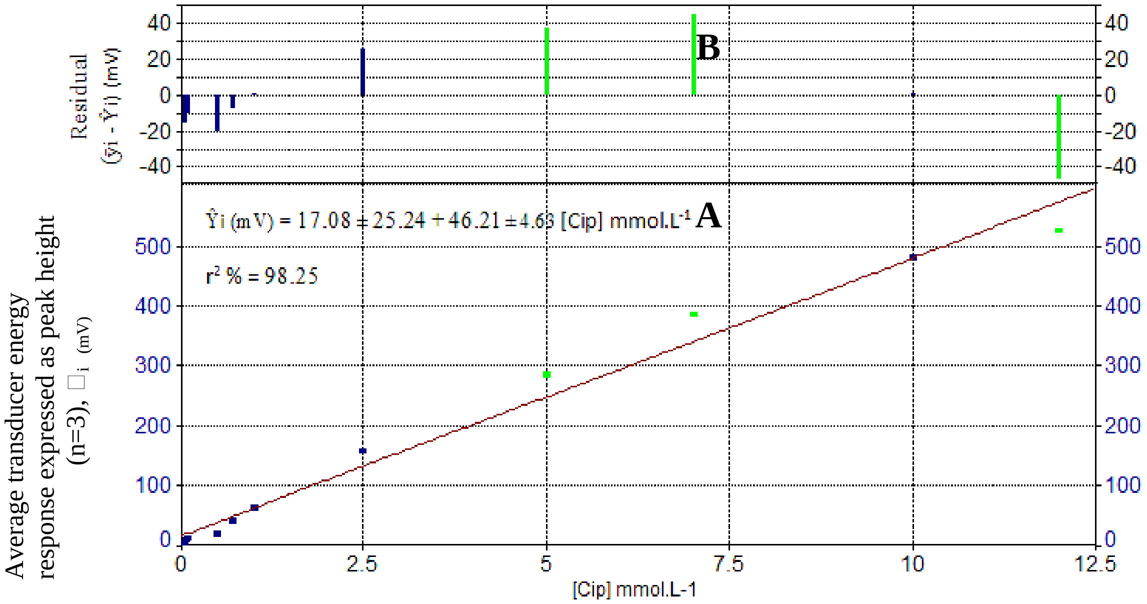


Figure 11: Calibration graph for the variation of Cip concentration on: A-transducer energy response expressed by linear equation using Ayah $3S_{BGR}$x3-3D solar cell CFIA micrphotometer, B- residual (□i – Ŷi), □i: practical value, Ŷi: estimate value.

Limit of detection (L. O. D):

Three different approaches were used for measuring L.O.D: gradual dilution of lowest concentration in the calibration graph, or detection based on the numerical value of slope or from the linear regression plot. Table 9 tabulated all these calculation value of detection limit for 156 µL sample volume.

Table. 9: summery of limit of detection based on different approaches at 156 µL sample volume.

Gradual dilution for minimum concentration	Based on the value of slope $X = 3S_B / slope$	Theoretical value from linear equation Ŷi (mV) = Y_B+3S_B
0.300 µg	39.07 µg	109.95 µg

S_B: standard deviation of blank solution. , X= value of L.O.D based on slope.
Y_B: average response for the blank solution (equivalent to intercept in straight line equation).

Repeatability:

The repeatability of measurement and the efficiency of homemade Ayah $3S_{BGR}$x 3 – 3D solar cell CFIA microphotometer were studied at fixed concentrations of Cip was used, mainly two concentration were used 7, 12 mmol.L^{-1}, using the optimum parameters. The repeated measurements for nine and eight successive injections were measured and obtained results were tabulated in table. 10 which shows that the percentage relative standard deviation was less than 0.6%, while figure 12 shows a kind of response-time profile for the used concentrations.

Tablet. 10 : Repeatability of Cip results obtained for the formation of colored species from Cip-Ce(IV) sulfate –H3O^{+} system.

[Cip] mmol.L^{-1}	no. of injection	Average transducer energy response expressed as peak heights □i (mV)	RSD %	Confidence interval of the average response 95 % confidence □i (mV)±$t_{0.05/2,\ n-1}$ $\sigma_{n-1}/\sqrt{n}$
7	9	385.33	0.519	385.33 ± 1.57
12	8	527.00	0.536	527 ± 2.43

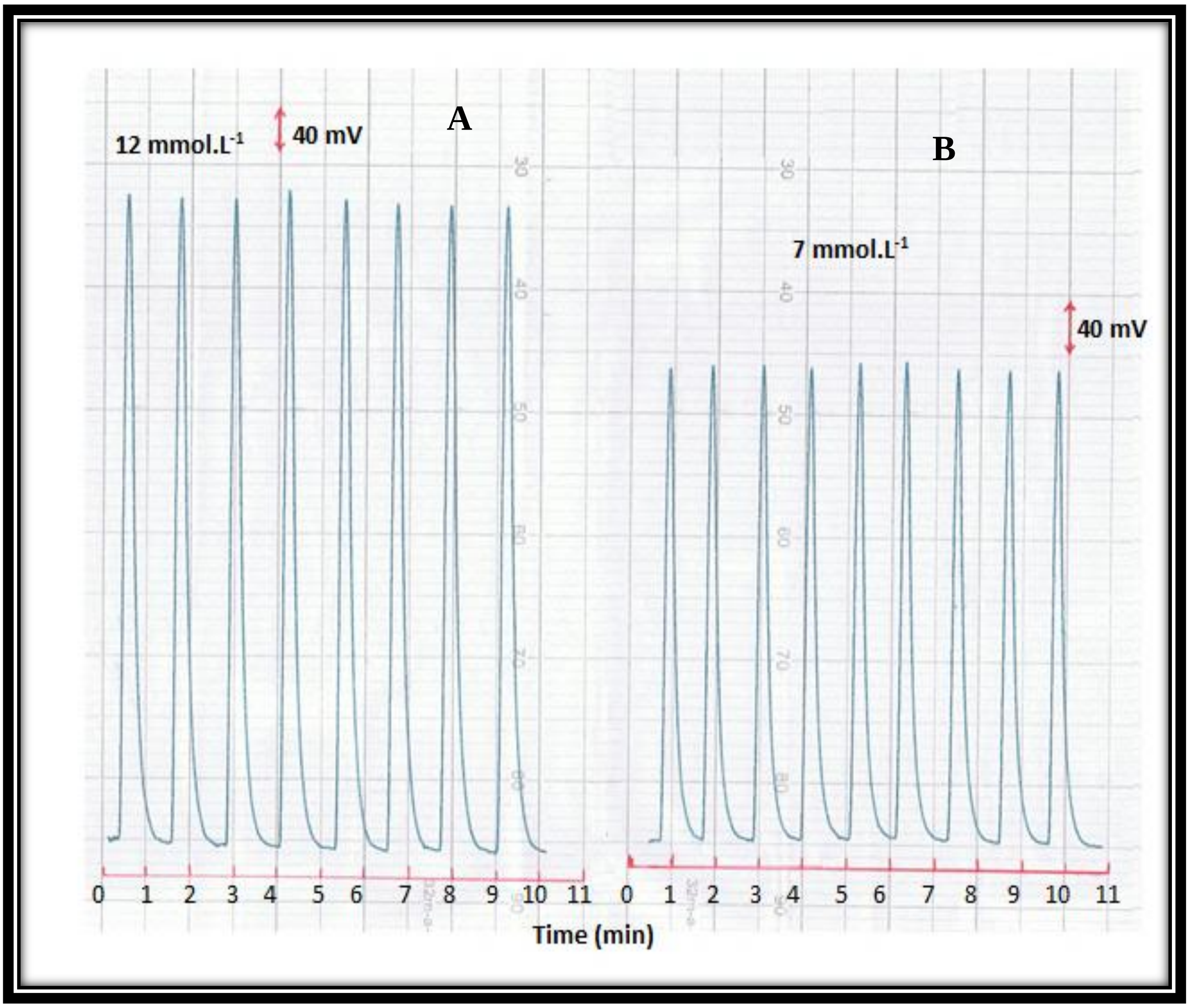


Figure 12: A Profile of successive repeatability measurements of Cip (A: 12, B: 7, mmol.L^{-1}) using Ayah 3S$_{BGR}$ x3S-3D solar cell CFIA microphotometer.

Analysis of pharmaceutical preparation:

The established method was used for the determination of Cip in three different kind of 500 mg Cip from three different well known drug manufactures (Ciprova- lupin- India) , (Ciproxene- Medical bahri- Syria), and (Cipropharma- Pharma international- Jordon) using Ayah $3S_{BGR}$ x 3S – 3D solar cell CFIA microphotometer and was compared with classical spectrophotometric method via the measurement of λ_{max} at 551 nm . The standard addition method was applied by prepared a series of solutions from each pharmaceutical drug via transferring 0.5 mL, (50 $mmol.L^{-1}$) for the proposed method of each drug(Quated value-500 mg), to five volumetric flask (25 mL) , followed by the addition of (0 , 0.5 , 1.25 , 2.5 , and 3.5) from standard solution of Cip (50 $mmol.L^{-1}$) in order to have the concentration range from 0 – 7 $mmol.L^{-1}$, while transferring 0.25 mL (50 $mmol.L^{-1}$) for the classical method to the five volumetric flask (25 mL) followed by the addition of (0, 0.025, 0.035, 0.05, and 0.25 mL) from standard solution of Cip (50 $mmol.L^{-1}$) in order to have the concentration range 0 – 0.5 $mmol.L^{-1}$ for the preparation of standard additions calibration plot. The measurements were conducted by both methods.

Results were mathematically treated for standard additions method. The results were tabulated in Table. 11 at confidence interval 95 % [32].

Paired t – test was used as shown in Table. 12 which show a comparison – treatment of data were subjected at two different paths.

First approach: comparison of newly developed method with official quoted value (500 mg) as shown in table 12 column (4) by calculated t – values with individual t – test. It was found that there was no significant difference between the quoted value of each individual company with calculated t – value at 95 % confidence interval as the calculated t – value is less than critical tabulated t – value .

Second approach: comparison of obtained results from both methods with neglecting the difference of origin. It was noticed that there were no significant difference between two methods as shown in column 8 at 95%. Table no (12) shows results of paired t – test for the new adopted method (Ayah $3S_{BGR}$ x 3 –3 D solar cell CFIA micro photometer) and classical method (UV – Vis spectrophotometric) for the determination of Cip in pharmaceutical preparation.

Table no. 11: results for the determination of Cip in pharmaceutical preparation using standard addition, with two methods Ayah 3S$_{BGR}$ X3 - 3D solar cell CFIA microphotometer and UV-Vis spectrophotometric method.

Sample no.	Commercial name, quoted Active ingredient, company, country	Confidence interval for the average weight $\overline{w}\pm1.96\,\sigma_{n-1}/\sqrt{n}$ at 95% (g)	Sample weight equivalent to 1.9291 g (50 mmol.L^{-1})of the active ingredient (g)	Theoretical content for the active ingredient at 95% (mg)	Equation of standard addition curve at 95% for n-2 $\hat{Y}_{(mV)}=a\pm s_a t+b\pm s_b t[x]$	r r^2%	Practical conc. (mmol.L^{-1}) and what is equivalent of active ingredient (mg)	Practical content of active ingredient at 95% (mg)	Efficiency of determination (%Rec)
					AYAH 3S$_{BGR}$ x3-3D Solar cell CFIA microphotometer				
					UV - Vis spectrophotometer				
1	Ciprova, 500 mg Lupin, India	0.7801±0.0027	3.010	500±1.73	48.82±32.42+49.54±8.05[x]	0.9960 99.22	49.5 1909.8	495± 3.709	99.00%
					0.24±0.02+0.48±0.08[x]	0.9961 99.24	49.6 1913.7	495.973±4.340	99.19%
2	Ciproxene, 500 mg, M.Bahri, Syria	0.771223±0.0075	2.97553	500±4.86	53.08±14.58+53.13±3.63[x]	0.9992 99.86	49.96 1928	499.72± 2.169	99.94%
					0.24±0.02+0.49±0.10[x]	0.9927 98.55	49.0 1890.5	489.866±6.641	97.97%
3	Cipropharm, 500 mg, pharma international, jordan	0.774584±0.0062	2.9884	500±6.82	61.02±9.80+60.19±2.42[x]	0.9997 99.95	50.5 1948	504.916±3.571	100.98%
					0.30±0.03+0.59±0.16[x]	0.9900 98.01	50.8 1932.1	500.934±3.448	100.19%

$\hat{Y}i$ = estimated value for energy transducer response (mV) or absorbance, $t_{0.025,\infty}$ = 1.96 at 95 %, X: [Cip] mmol.L^{-1}.
(0.5mL*), and (0.25mL**) , drawing volume for the proposed method and classical method respectively.

Table 12: paired t –test results for Ayah 3 S_{BGR} x 3S –3 D solar C F I A micro photometer (proposed method) with classical method using standard additions method for determination of Cip in pharmaceutical preparation.

Sample no.	A moment found $\bar{x}$ (mg) ± RSD % at 95 % , n = 3		$(\bar{x}-\mu)\sqrt{n}/\sigma_{n-1}$ Ayah $3S_{BGR}$x3-3D solar CFIA with Quoted (500 mg)		X d	$\bar{x}$ d	σ_{n-1}	$t_{cal} = \frac{\bar{x}d\sqrt{n}}{\sigma_{n-1}}$ at 95%	t_{tab} at 95%
	Proposed method Ayah $3S_{BGR}$x3S-3D solar cell CFIA microphotometer	UV-Vis method	t_{Cal}	t_{tab}					
1	495±3.709	495.973±4.340	$\lvert -3.76 \rvert$ <<	4.303	-0.937	4.297	5.402	1.378 <<	4.303
2	499.72±2.169	489.866±6.641	$\lvert -0.42 \rvert$ <<	4.303	9.854				
3	504.916±3.571	500.934±3.448	4.25 <<	4.303	3.976				

$t_{0.05/2\ ,\ n-1,} = 4.303$

Conclusion:

A spectrophotometric CFIA is proposed method for determination of Cip with application in the quality control analysis of pharmaceutical formulations. The method based on the oxidation of Cip by Ce(IV) sulfate in acidic medium forming pinkish red product . The experimental point of view, the manipulation is very simple and sequential measurement was permitted with sample frequency up to 30 samples per hour. The proposed method uses cheaper instrument and reagent. In this paper a more effective faster determination was achieved by Ayah $3S_{BGR}$ x 3-3D solar cell CFIA microphotometer , light emitted diode as source with a detection using solar cell . The standard additions method was used to avoid matrix effects. Also this method can be applied to micro determination of Cip in pure as well as in pharmaceutical preparation and over high sensitivity without the need for heating or extraction.

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