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

تقدير السايبروفلوكساسين في مستحضراته الدوائيه باستخدام تحليل الحقن الجرياني المستمر عن طريق قياس التعكريه (T_{180o}) وتأثير استطاره الضوء عند اتجاهين متعاكسين ($2N_{90o}$) باستخدام Ayah $4S_W$-3D-T_{180} -$2N_{90}$ -Solar - CFI المحلل

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Abstract

A simple and highly sensitive method for the determination of ciprofloxacin in pure and pharmaceutical preparation were developed by coupling the continuous flow injection analysis via turbidimetric (T_{180}^{o}) and scattered light effect at two opposite position ($2N_{90}^{o}$). Where it is based upon the formation of a yellowish –white precipitate for ion pair compound by using potassium hexacyanoferrate(III) in aqueous medium. The precipitate is measured via the attenuation of incident light and it's scattering in two opposite directions. Chemical and physical parameters were studied. The linearity of ciprofloxacin is ranged from 1 to 20 mmol.L^{-1}, with correlation coefficient r=0.9927, limit of detection (LOD) 0.55 mmol.L^{-1}($3S_B$)(S/N=3) for n=15 and the relative standard deviation for 4 mmol.L^{-1} ciprofloxacin solution is lower than 2% (n=6). Three pharmaceutical drugs were used as an application for the determination of ciprofloxacin. A comparisons were made between the newly developed method of analysis with the official method (uv-vis-spectrophotometry) of analysis using the standard addition method. It shows that there was no significant difference via the use of t- test at α=0.05(95% confidence) between the two methods. Therefore the newly developed method can be used of as an alternative method for the analysis of ciprofloxacin.

Key word: Ciprofloxacin, Spectrophotometry, Turbidity & Nephelometry, Flow injection analysis

الخلاصة

استحدثت طريقة بسيطة وسريعة لتقدير سايبروفلوكساسين بشكله النقي او على هيئة مستحضراته الدوائية من خلال اقتران تقنية الحقن الجرياني المستمر مع قياس التعكريه والاستطاره. وتستند هذه الطريقة على تشكيل راسب ابيض مصفر للمزدوج الايوني بين السايبروفلوكساسين و العامل المرسب $K_3[Fe(CN)_6$ في الوسط المائي. حيث يتم قياس الراسب عن طريق توهين الضوء الساقط وكذلك استطارته عند زاوية قائمه وابتجاهين متعاكسين . وبعد دراسة العوامل الكيميائية والفيزيائية للحصول على استجابات مثلى, تراوحت الخطية للسايبروفلوكساسين بين (1- 20) ملي مول.لتر$^{-1}$ مع معامل الارتباط r= 0.9927 وحدود الكشف (L.O.D) 0.55 ملي مول.لتر$^{-1}$ ($3S_B$) (3 = S/N) ولـ n = 15 كما ان الانحراف القياسي المئوي اقل 2% لتركيز 4 مللي مول.لتر$^{-1}$سايبروفلوكساسين ل(n = 6). تم تطبيق هذه الطريقة بنجاح لتحديد السايبروفلوكساسين في ثلاثة عقاقير دوائية. كما قدمت مقارنة بين الطريقة المستحدثة مع الطريقة التقليدية للقياس الطيفي (طريقة مطيافية الأشعة فوق البنفسجية) باستخدام طريقة الاضافات القياسية وذلك من خلال استخدام اختبار- t وبين عدم وجود فرق جوهري عند =0.05 α وحدود ثقه 95% بين الطريقتين وبالامكان استخدام الطريقه المستحدثه كطريقة بديلة لتقدير السايبروفلوكساسين.

Introduction

Ciprofloxacin hydrochloride(figure no.1), a fluoroquinolone, is the monohydrochloride monohydrate salt of 1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinolinecarboxylic acid, Figure no. 1 is a fluoroquinolone-type antibiotic agent. It exhibits broad spectrum antimicrobial activity against Gram-positive and Gram-negative bacteria such as Pseudomonas aeruginosa, Streptococcus faecalis, Staphylococcal aureus, and Enterobacter aerogenes [1,2]. It is used in the treatment of a wide range of infectious diseases. [3] Ciprofloxacin is also one of the antibiotics approved by the FDA for patients who have been exposed to the inhaled form of anthrax. Its mode of action depends upon blocking bacterial DNA replication by binding itself to an enzyme called DNA gyrase, thereby preventing the enzyme's ability to untwist the DNA double helix, which is required for DNA replication.[4]

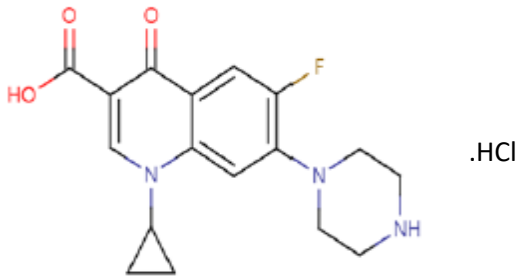

Fig.1: Structure of Ciprofloxacin

Turbidimetry is the measurement of the degree of attenuation of a radiant beam incident on particles suspended in a medium. Nephelometry is the measurement of the light scattered by suspended particles, the measurement usually being made at 90° to the incident beam.[5]The development of the first analytical turbidimeter was in the 1960s and the fundamental optical technology remained unchanged until the mid-1980s. Since then, instrument design technology has advanced dramatically and many new designs emerged . These new designs have evolved to address many of the traditional interferences associated with turbidity. Because different technologies (such as light sources and detector design) have been used to compensate or eliminate interferences such as color, bubbles, stray light, absorption, and path length, it is often difficult or impossible to compare measurements[6].The first report in using the turbidimetry in the flow-injection system for determination of sulfate by monitoring the barium sulfate suspension. In spite of the routine use of flow-injection system with turbidimetric detection for the determination of inorganic species in plants and water [7] . Applications to pharmaceutical products were limited. [8]

Several analytical methods have been developed for the determination of ciprofloxacin. In lecturer review , ciprofloxacin was determined by high performance liquid chromatography (HPLC) [9-23], voltammetry [24-26] , Spectrofluorimetric method,[27-29],Biosensors [30,31] , HPLC–MS/MS [32- 35], Solid phase spectrophotometry[36], micro emulsion electro kinetic chromatography (MEEKC) method [37],Microbiological turbidimetric method[38] Spectrophotometry[39-46] , Micellar liquid chromatographic (MLC)[47] electrophoresis[48], flow injection UV spectrophotometric [49,50], flow injection chemiluminescence (CL)[51-54], thin-layer chromatography is established, with micelle solutions as mobile phases(Micelle TLC–Fluorimetry)[55]., the Rayleigh light scattering technique [56],Derivative spectrophotometertric [57] , and Fourier transform infrared spectrometric (FTIR).[58]

This research work describes a methodology for determination of ciprofloxacin in pharmaceutical formulations based on the continuous flow injection analysis(FI) via turbidimetric and scattered light at two opposite position .This method uses $K_3[Fe(CN)_6]$ as a precipitating reagent in aqueous medium . The precipitate is measured via the attenuation of incident light and scattering of the incident light in +90° and -90° angle. The output signal was recorded as an

analytical response via time for each signal concentration level. The obtained results were compared with the uv-vis spectrophotometric method.

Experimental

Chemicals

All chemicals were used of analytical-reagent grade while distilled water was used to prepare the solutions. A standard solution of ciprofloxacin (CIP) ($C_{17}H_{18}FN_3O_3.HCl.H_2O$, molecular weight 385.8, SID, 0.1mol.L^{-1}) was prepared by dissolving 3.858 g in 100ml distilled water. A stock solution Potassium hexacyanoferrate(III) ($K_3[Fe(CN)_6]$, molecular weight 329.24, 0.1mol.L^{-1}):was prepared by dissolving 3.2924 g in 100 ml of distilled water. A cerium (IV) sulphate solution ($CeSO_4$, molecular weight 236, 0.025mol.L^{-1}) was prepared by dissolving about 10 g in 1.0 M sulphuric acid and diluting to 1L with the same acid . Sulphuric acid solution (98% H_2SO_4, 1.84g.ml^{-1}, 5 mol.L^{-1}) was prepared by adding 272 ml of sulphuric acid to 728 ml water with constant cooling. A 500 mg.L^{-1}methyl orange solution was prepared by dissolving 59 mg of the dye (dye content 85 %) in water and diluting to the mark in a 100 ml calibrated flask and filtered. This was diluted 10-fold to obtain a working concentration of 50 mg. L^{-1}. Hydrochloric acid solution was prepared by diluted 88.25 ml of 35% HCl (1.18 g.ml^{-1}, BDH, 1mole.L^{-1}) with distilled water in 1L calibrated flask. Aqueous solutions of nitric acid was prepared by diluting 64ml HNO_3 (70%,1.42g.ml^{-1}, BDH, 1mol.L^{-1}) to 1L with distilled water.

Sample preparation

The procedure was adopted by selecting thirteen tablets. The tablets were weighed, crashed, and grinded by pestle and mortar until fine powder +200 meshes. A 0.05 mmol.L^{-1} solution was prepared by weighing an amount equivalent to (0.963g) active ingredient for each pharmaceutical preparation. The powder was dissolved in distilled water followed by filtration to remove any undissolved residue. The filtrate was made up to 50ml with distilled water and further solutions were prepared by appropriate dilution.

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[59] .Which uses four white snow LED for irradiation of the flow cell at 2mm path length. . Three solar cell were used as a detector for collecting signals via travelling of sample for 40mm length . The readout of the system composed of x-t potentiometric recorder (KOMPENSO GRAPH C-1032) SIEMENS (Germany) or digital AVO-meter (auto range)(0.00-2000mV) (China). Spectrophotometric readings under batch conditions were made by means of a Shimadzu (Japan) UV-1800 double-beam spectrophotometer and quartz cuvette with an optical path length of 10 mm. Figure(2) is shown flow gram for ciprofloxacin determination.

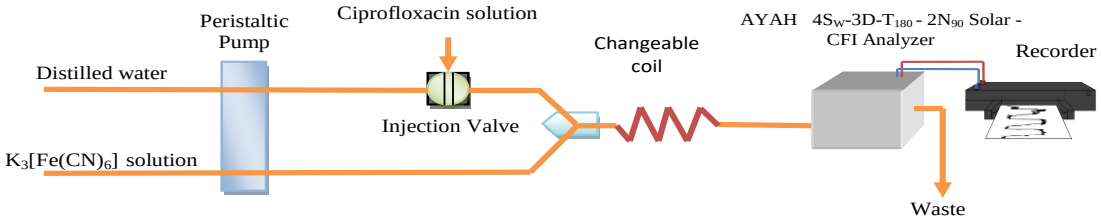

Fig. 2: Flow gram for ciprofloxacin

Methodology

Flow injection system for the reaction of CIP-$[Fe(CN)_6]^{3-}$ to form precipitate as an ion pair is composed of two lines as shown as in Fig. no. 2 . The first line is the carrier stream (distilled water) at 2.7 $ml.min^{-1}$Flowrate which leads to the injection valve to carry ciprofloxacin sample of 130 µl; while the second line supplies $K_3[Fe(CN)_6]$ solution (4 $mmol.L^{-1}$) at (2.9 $ml.min^{-1}$). Both line meet at a junction (Y- junction), with an outlet for reactants product of ion pair by using Ayah $4S_W$-3D-T_{180} - $2N_{90}$ - Solar - CFI Analyser four successive instantly signals can be recorded through .The four signals represent turbidity (i .e attenuation) and reflection of incident light at two opposite perpendicular direction. While the fourth response represent the algebraic sum of both reflected light at two opposite direction. Figure no.3 is shown a probable proposed mechanism of ion pair for system CIP-$[Fe(CN)_6]^{3-}$ in aqueous medium[60].

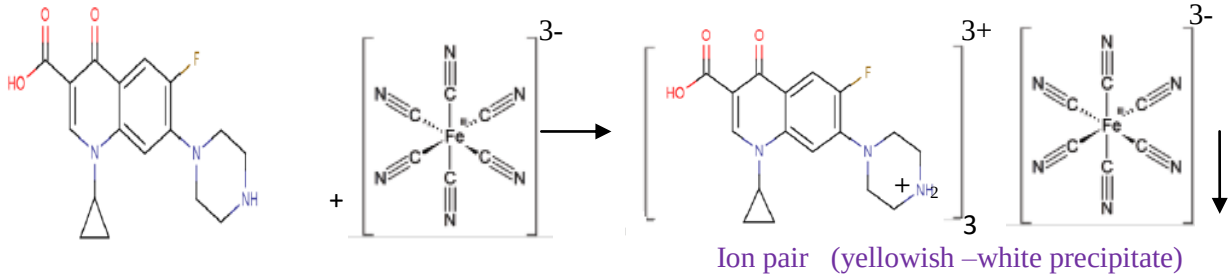

Fig. 3: Probable proposed mechanism of reaction between ciprofloxacin and $K_3[Fe(CN)_6]$

Results and Discussion

Variable optimization

The variables influencing the performance of the method were optimized .The optimum values were selected depending on sensitivity and reproducibility of signals. The chemical parameter (mainly concentration and pH for the reaction medium) .In addition, physical parameters (intensity of incident light p^o ,volume of coil , flow rate, sample volume and purge time) were studied.

Effect of $K_3[Fe(CN)_6]$ concentration

A series of the precipitating reagent ($K_3[Fe(CN)_6]$) solutions (1- 8 $mmol.L^{-1}$) were prepared. 10 $mmol.L^{-1}$ of ciprofloxacin was used 100 µl sample volume at 2.7 $ml.min^{-1}$ flow rate and the intensity of incident light of total four LEDs 1400 mV. The total results obtained was tabulated in Table no. 1; represented via four x-t potentiometric recorder. It can be seen that an increase in $K_3[Fe(CN)_6]$ causes an increase in the incident light due to the reflections of the light on particles surfaces. While there is an increase of at N_L followed by a nearly constant response .The same behavior is observed in N_R as shown in Fig. (4) . 4 $mmol.L^{-1}$ $K_3[Fe(CN)_6]$ concentration was chosen as the optimum concentration that used for further experiments.

Table no. 1: Effect of $K_3[Fe(CN)_6]$ on the measurement of attenuation of incident light as well as reflection of light at two opposite position also algebraic sum of them

$K_3[Fe(CN)_6]$ 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 $T_{(0-180)}$	Scattering of light in left N_L (+90)	Scattering of light in right N_R (-90)	The final outcome of the scattering of light $N_{(L+R)}$ (±90)
1.0	80±1.305	36±0.788	4±1.033	20±1.943
2.0	247±1.722	131±0.000	8±0.450	65±1.972
3.0	320±0.986	140±0.236	12±0.342	76±0.000
4.0	400±0.953	176±0.178	16±0.793	96±1.751
5.0	405±0.325	164±1.673	18±1.024	88±0.853
6.0	392±0.589	160±1.954	16±1.667	85±0.564
7.0	395±0.872	156±0.000	17±1.457	84±0.675
8.0	379±1.149	155±0.342	18±0.336	86±0.068

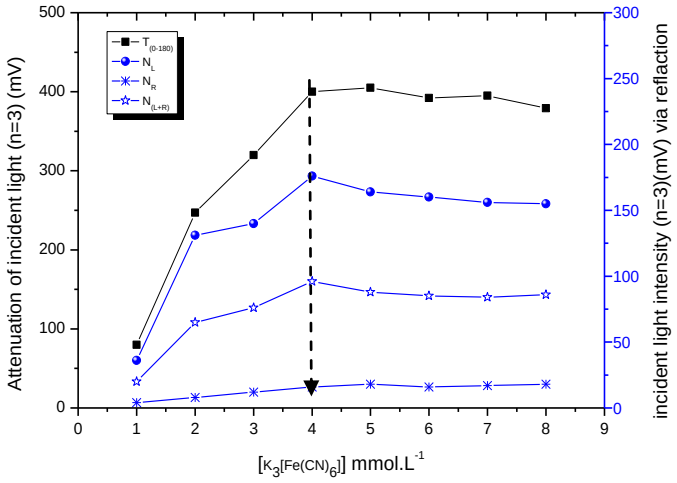

Fig. 4: Influence of $K_3[Fe(CN)_6]$ on attenuation of incident light, scattering of light at two opposite directions and their total outcome.

Influence of pH for the reaction medium

The effect of different reaction medium on the sensitivity in general was studied. Different acid solutions (0.1mol.L^{-1} for each of HCl, H_2SO_4 and HNO_3) were prepared from Fig. no.5 can be seen that an increase in sensitivity of response in aqueous medium of carrier stream compared with the scattering of incident light due to the decrease in the amount of precipitate due to solubility in acidic medium .Also an increase of at N_L and The same behavior is observed in N_R.Aqueous medium (i.e.distilled water) was regarded as the optimum medium for the use and was chosen for further use.

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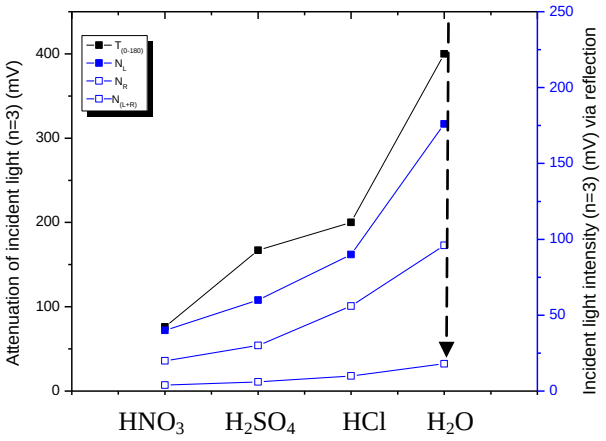

Fig. 5: Influence of pH of medium on the measurements of turbidity as well as reflection of light at two opposite position and algebraic sum of them.

Incident light intensity

Variable intensity of light source was used 0.6 – 1.85 volt by variation of light intensity channel in AYAH4S_W-3D-T_{180} -2N_{90}-Solar GFI Analyzer operation where read by AVO-meter. The results tabulated in table 2 shows that an increase in the attenuation of incident light ; also this increases will extent to the scattering of light in two ways The intensity of (1.47 volt) was selected as the optimum voltage that can be supplied to give a better reproducible outcome as shown in Fig. 6.

Table no. 2: Effect of Intensity of light source (LED) (P^o) on attenuation of incident light, scattering of light at two opposite position and the algebraic sum of them.

Intensity of source (Volt)	Type of measurement $\bar{y}_i \pm t_{0.05/2}\ \sigma_{n-1}/\sqrt{n}$ (n=3) (mV)			
	Attenuation of incident light $T_{(0-180)}$	Scattering of light in left N_L (+90)	Scattering of light in right N_R (-90)	The final outcome of the scattering of light $N_{(L+R)}$ (±90)
0.6	375±0.556	32±1.347	4±0.000	16±0.352
0.9	490±1.680	56±1.889	8±1.057	32±0.735
1.1	440±1.235	88±1.538	10±0.000	50±1.432
1.35	380±0.000	132±1.675	12±1.242	72±1.584
1.47	390±0.533	176±0.989	16±1.113	92±0.649
1.65	380±1.067	240±1.067	24±1.563	120±0.938
1.75	360±1.246	320±1.442	26±1.870	160±0.005
1.85	400±1.564	360±0.000	28±0.584	176±0.084

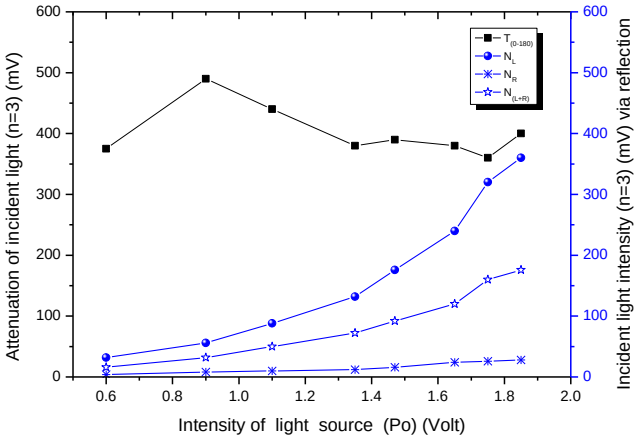

Fig. 6: Effect of variation of intensity of light source on the measurements of turbidity as well as reflection of light at two opposite position and algebraic sum of them.

Reaction coil length

The effect of reaction coil length was evaluated through the use of a coil length from 10 to 50cm. This range of length comprises a volume of 314 to 1570 µl. The table no.3 shows clearly that length of 10cm (341µl) reaction coil will serve as a more reproducible and for more sensitive measurements above which a slight decrease was observed probably due to the dispersion of the sample zone. Fig. no.7-A shows the effect of reaction coil length on attenuation of incident Light, scattering of light at two opposite directions and the algebraic sum of them. Fig. no.7- B shows response profile of optimum coil length that was selected for further works.

Table no.3: Effect of coil volume on attenuation of incident light, scattering of light at two opposite directions and total outcome of them via reflection.

No. of measurement	Length of coil (cm) Diameter(mm)	Volume of coil (µL)	Arrival time of injected sample to nubble of the measure (Sec.)	Dilution factor (D.F)	Type of measurement $\tilde{y}_i \pm t_{0.05/2}\ \sigma_{n-1}/\sqrt{n}$ (n=3) (mV)			
					Attenuation of incident light $T_{(0-180)}$	Scattering of light in left N_L (+90)	Scattering of light in right N_R (-90)	The final outcome of the scattering of light $N_{(L+R)}$ (±90)
1	10(2mm)	314	12	4.14	416±0.772	160±0.580	16±1.233	88±1.067
2	50(0.5mm)	393	16	4.42	408±0.938	148±0.000	12±0.000	80±1.086
3	20(2mm)	628	19	7.28	440±0.874	183±0.963	22±0.000	108±0.573
4	30(2mm)	942	18	9.42	432±1.005	180±1.438	24±1.057	100±0.000
5	40(2mm)	1252	22	13.52	420±1.743	192±0.000	20±0.874	104±1.084
6	50(2mm)	1570	24	16.70	412±1.229	196±0.864	18±1.234	100±0.692

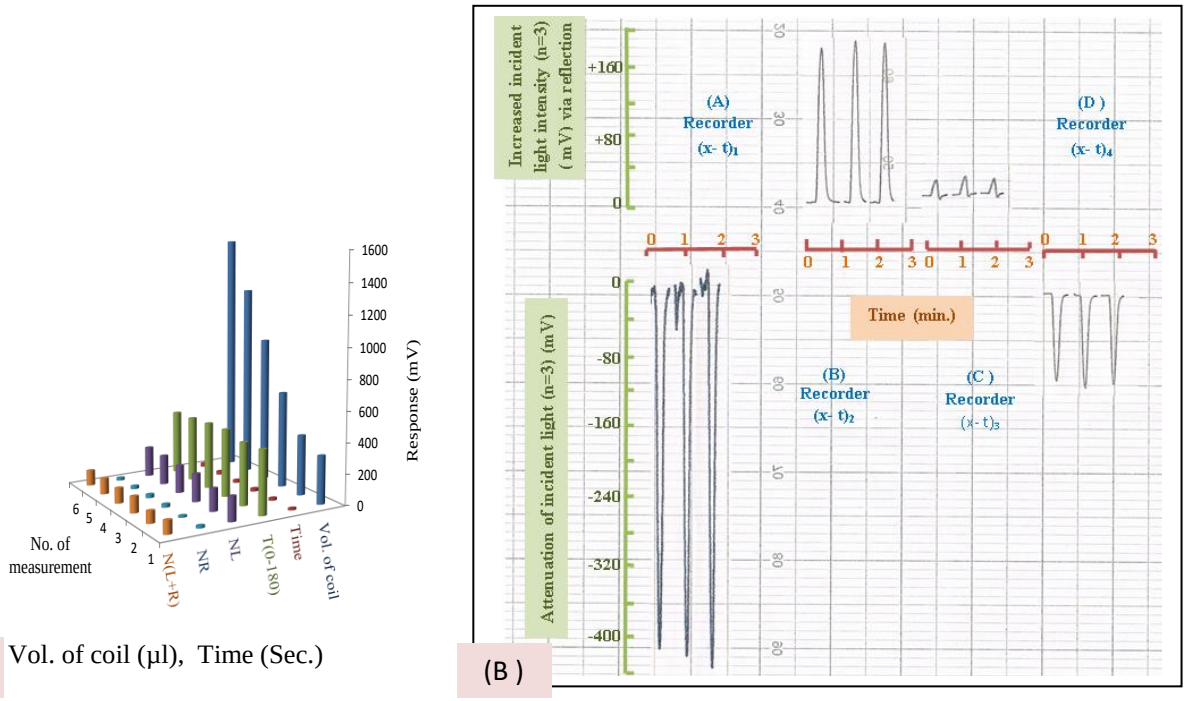

Fig.**7** Effect of reaction coil length on :
(A) Attenuation of incident Light, scattering of light at two opposite directions and the algebraic sum of them.
(B) Response profile of optimum coil length

Flow rate

The influences of the flow rate on the measurement were studied. Carrier flow rates ranging from 0.75 to 3.9 ml.min^{-1} were assayed with the aim to evaluate their effect on the peak height and repeatability of the analytical data. Figure (8) shows that at low flow rate there is an increase in dispersion and dilution. Maximum sensitivity was obtained at 2.7 ml. min^{-1} flow rate of carrier that can be supplied to give a better reproducible outcome.

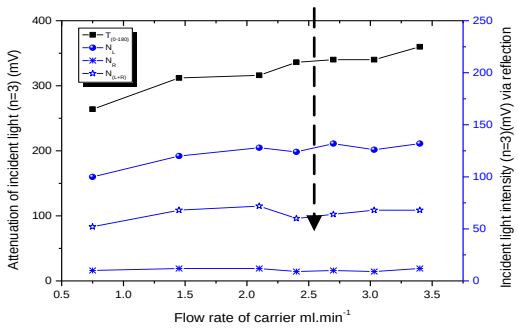

Fig. 8: Influence of variation of flow rate on the measurements of turbidity as well as reflection of light at two opposite position and algebraic sum of them .

Sample volume

The injected volume of sample was varied in the range 20 – 140 µl by changing the length of the sample loop in the injection valve, while the other variable remained fixed. An increasing in the injection volume led to a significant increase in sensitivity, more perceptible than low volumes as shown in Fig.(9-A) which shows that the optimum sample volume of 130 µl gave regular responses

to the attenuation of incident light and scattering of light (±90). Using sample volume > 130µl even though it gave a slight response but it was characterized with the width that could be attributed to a long time duration of the precipitate ion pair formed as illustrated in fig. (9-B).

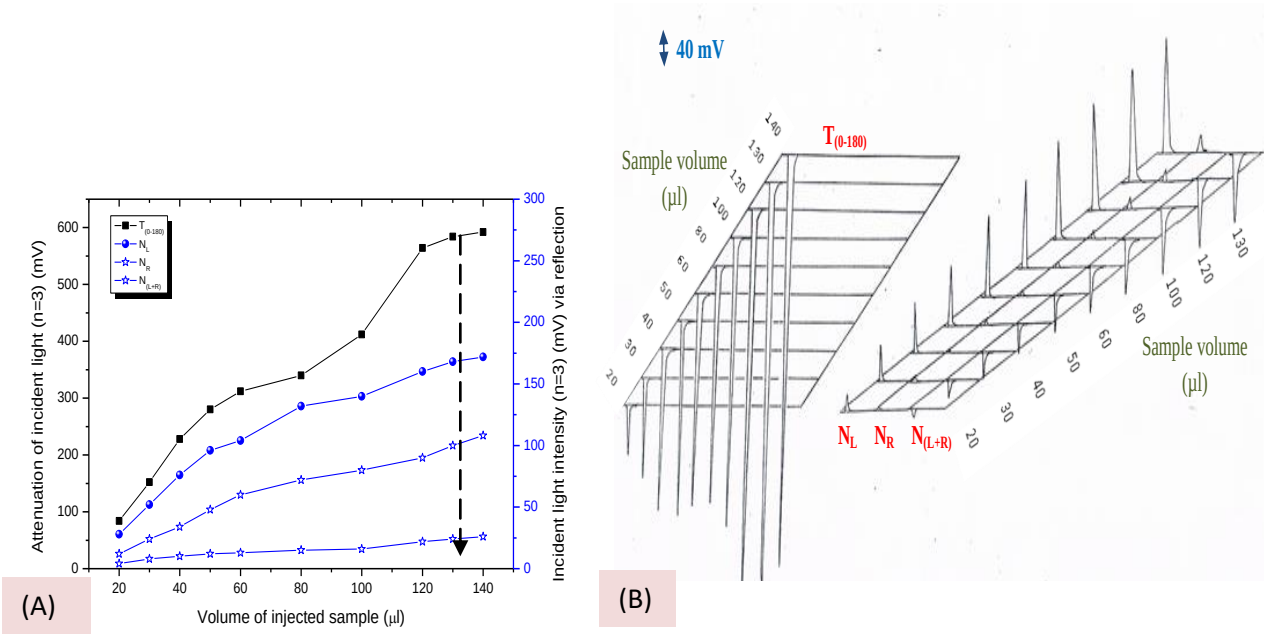

Fig. 9 Effect of volume sample on: (A) Attenuation of incident Light, scattering of light at two opposite directions and the algebraic sum of them via reflection .(B) Response profile.

Purge Time

Allowed permissible time for the sample to be injected via the carrier stream was studied. The effect on the response and it is sensitivity was followed using the optimum physical and chemical parameters achieved in previous sections. Allowed time of 5, 10,15 ,20 ,25 and 30 seconds and open time were used for this study. It can be seen from the Fig.10.A,B that there is an increase in the response with increasing the allowed permissible time. 30 seconds was found to be the optimum purge time.

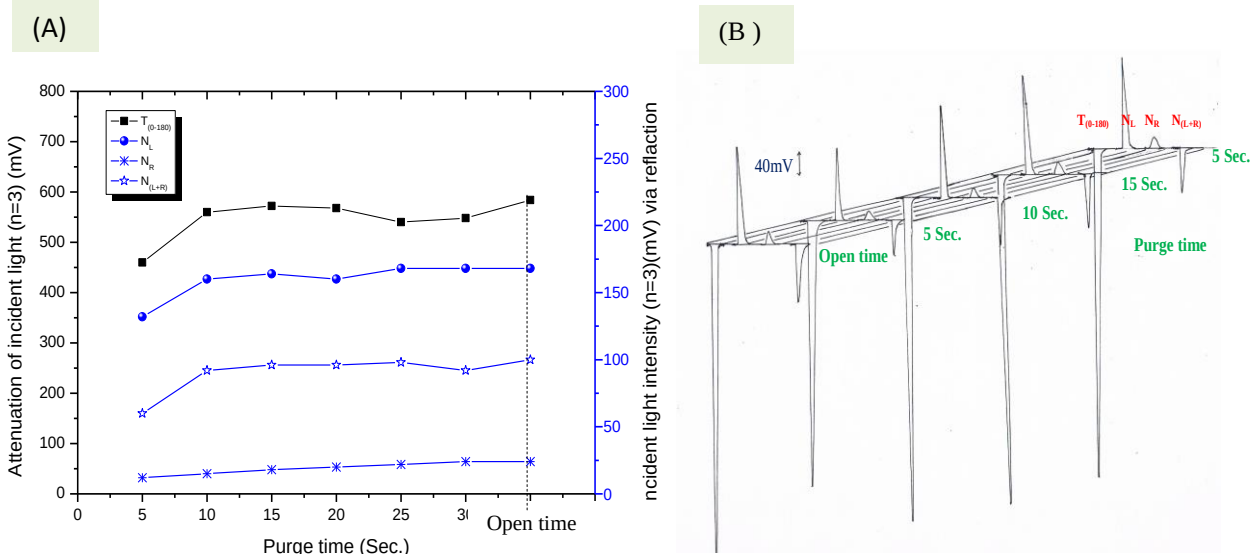

Fig. 10 Effect of of purge time on: (A) Attenuation of incident Light, scattering of light at two opposite directions and the algebraic sum of them via reflection . (B)

Calibration curves and statistical parameters

When chemical and physical parameters were studied, the calibration curves of continuous flow injection analysis via attenuation of incident light, scattering of light at two opposite position method were estimated. A series 1-20 mmol.L^{-1} ciprofloxacin solution were prepared .Table no. 4 illustrated the results obtained.

Table no. 4: Summary of linear regression equation [61, 62] for estimate of ciprofloxacin by FIA method

Type of measured	No. of measurement	Range of [CIP] mmol.L^{-1}	ŷ(mV)=a± S_at+ b± S_bt[X] at confidence interval 95%, n-2	r r^2%	t_{tab} at 95% confidence interval, n-2	$t_{cal} = \frac{\lvert r\rvert\sqrt{n-2}}{\sqrt{1-r^2}}$
$T_{(0-180)}$	15	1-20	-32.55±32.92+37.98±2.74[X]	0.9927 98.56	2.160<<29.826	
N_L	11	4-17	-21.61±18.30+16.08±1.61 [X]	0.9912 98.26	2.262<<22.542	
N_R	11	4-17	-5.89±2.58+2.18±0.23[X]	0.9906 98.13	2.262<<21.732	
$N_{(L+R)}$	11	4-17	-46.06±23.12+16.88±2.04[X]	0.9874 97.50	2.262<<18.735	

ŷ= Estimated response (mV) for (n=3),[x] = ciprofloxacin conc. (mmol.L^{-1}), r= correlation coefficient, r^2%: linearity percentage

Limit of detection for CPI was calculated through three methods as tabulated in table no.5 at injected sample volume of (130µl) . Also L.O.Q was reported.

The value of RSD% for some selected concentration of CIP tabulated in table no. 6. This low percentage of relative standard deviation (less than 2%) indicate a reliable measurement can be achieved using this method. Figure no. 11 is shown response profile of repeatability at 4 and 12 mmol.L^{-1} respectively.

Table no. 5: limit of detection of ciprofloxacin 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 $Ý=Y_B+3S_B$	Practically based on gradual dilution for the minimum concentration	L.O.Q $Ý=Y_B+10S_B$
0.1007 mmol.L^{-1}	0.1004 mmol.L^{-1}	0.5500 mmol.L^{-1}	0.3346 mmol.L^{-1}

X= value of L.O.D based on slope, S_B=standard deviation of blank, Y_B=Average response for blank, L.O.D =limit of detection, L.O.Q. =limit of quantitate

Table no. 6: The repeatability of ciprofloxacin at optimum parameters.

[CIP] 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) $\bar{y}_i \pm t_{0.05/2}\ \sigma_{n-1}/\sqrt{n}$
4	$T_{(0-180)}$	92.00	1.160	1.26	92.000±1.315
	N_L	29.33	0.082	0.28	29.333±0.093
	N_R	5.570	0.034	0.61	5.571±0.039
	$N_{(L+R)}$	15.50	0.223	1.44	15.500±0.253
12	$T_{(0-180)}$	330.00	1.790	0.54	330.00±2.03
	N_L	191.50	1.500	0.78	191.500±1.70
	N_R	21.00	0.089	0.42	21.000±0.101
	$N_{(L+R)}$	101.33	1.632	1.61	101.333±1.85

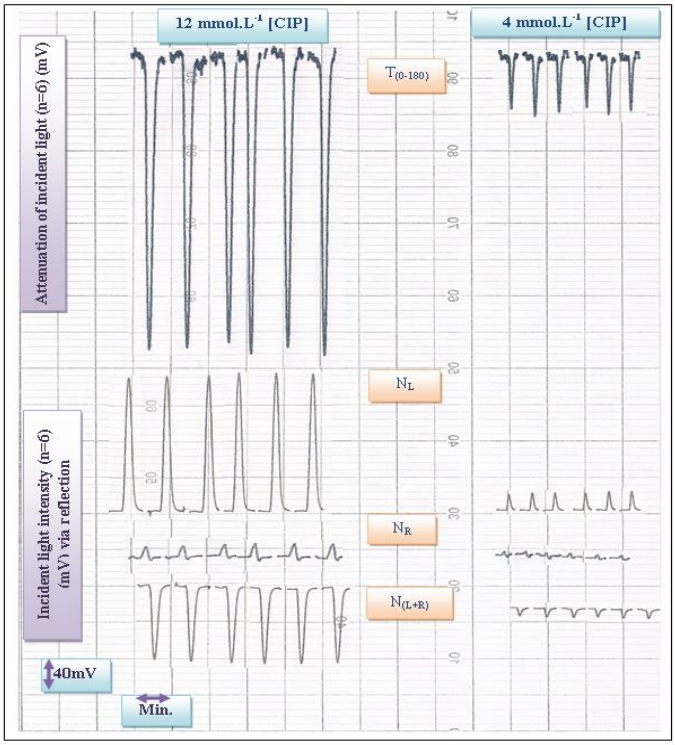

Fig. 11: Response profile of repeatability to attenuation of incident light, scattering of light at two opposite directions and the algebraic sum of them via reflection

Analysis of pharmaceutical preparation

Three different samples of pharmaceutical preparations (Ciprofloxacin, Ciprofloxacin Tablets USP, Cipropharm) were used. The CFIA via turbidity (T_{0-180}) and scattered light at two opposite location ($2N\pm_{90}{}^{o}$) method (new method) using Ayah $4S_W$-3D-T_{180} - $2N_{90}$ - Solar - CFI Analyzer achieved in this work and was compared by uv-vis spectrophotometry method [63] via measurement of λ_{max} at 520nm, linear calibration curve was obtained for the concentration range of 0.1-4 mmol.L^{-1}, correlation coefficient of 0.9950 was obtained while limit of detection was 0.06 mmol.L^{-1}. The preparation of standard addition calibration plot to uv-vis –spectrophotometry was prepared by different aliquots of 0 , 0.25, 0.5, 1.0, 1.5 and 2.0 ml from 15mmol.L^{-1} in order to have a concentration range of zero to 3.0 mmol.L^{-1} these solutions were transferred into a series of 10 ml calibrated flasks and the total volume was adjusted to 4.0 ml by distilled water. To each flask 0.5 ml of drug (20 mmol.L^{-1} CPF) were added, 1ml of 5 mol.L^{-1} sulphuric acid and 1ml of 1mol.L^{-1} cerium (IV) sulphate solutions, mixed well and the flasks were kept a side for 10 min. Then, 1 ml of methyl orange solution (50 mg. L^{-1}) was added. The volume was diluted to the mark with water and mixed. The absorbance of each solution was measured at 520 nm against a water blank after 5 min.

While the standard addition method for the new method that was applied by preparing a series of solution from each pharmaceutical drug by transferring 1ml to each of the five volumetric flask (10 ml), followed by the addition of 0, 1.0 , 1.8, 2.4 and 3ml of 40mmol.L^{-1} standard solution of ciprofloxacin in order to have the concentration range from 0 -12 mmol.L^{-1}.To each flasks were added 1ml of drug (50 mmol.L^{-1} CPF)The measurements were conducted by both methods. Results were mathematically treated for standard addition method. The results were tabulated in table no. 7,t-test was used as shown in table no.8 .A comparison was made between the FIA - turbidimatric-nephelometric method(FI- T&N)with officially used British pharmacopeia.The obtained values suggest that there is no significant difference between Quote and calculated t-values .There for on this basis T&N method can be regarded as an alternative method for the assessment of drug. In addition between uv-vis method with Quoted value (Claimed). It was found that there is no significant different between uv-vis method(official) and the Quoted value. Therefore any one of them can be used for comparison with FI- T&N method.

Table no. 7: Ciprofloxacin determination in pharmaceutical tablets by standard addition method using FI – T&N (depend on $T_{(0-180)}$) method & uv-vis spectrophotometric method

pharmaceutical drug company and Claimed content of active ingredient	Weight of sample (gm) that equivalence to (963.25 mg) of active ingredient to obtain 0.05mol.L^{-1} of ciprofloxacin in 50ml	Confidence interval of the mean $\overline{W} \pm t_{\frac{0.05}{2},(n-1)} \frac{\sigma_{n-1}}{\sqrt{n}}$ (gm)	$y^{\wedge}$(mV)=a± S_at+ b ± S_bt[X] at confidence interval 95%, n-2	r r^2%	Theoretical calculated active material (mg)	[CIP]mmol.L^{-1} measured				[CIP] mmol. L^{-1}	Practically found content of active ingredient mg	Efficiency of determination
			FI – T&N method			$T_{(0-180)}$	N_L	N_R	$N_{(L+R)}$	FI – T&N method		
			uv-vis sp. method							, uv-vis sp. method		
Ciprofloxacin BRISTOL U.K 500g	1.517	0.78484±0.00198	110±49.21+22.93±6.36[X]	0.98873 97.76%	500±2.408	4.82	4.52	4.51	4.51	4.82	482±5.45	96.4%
			0.3135±0.069+0.22±0.391 [X]	0.9982 99.66%						0.99	495±7.35	99%
Ciprofloxacin USP BAL-PHARAMA LIMITED India 500mg	1.412	0.73316±0.0039	79.65±27.62+15.9±3.56[X]	0.9925 98.52%	500±4.09	4.81	4.22	4.22	4.22	4.81	481±5.44	96.2%
			0.285±0.127+0.255±0.075 [X]	0.9874 97.50%						0.98	490±9.78	98%
Cipropharm Phama-international Jordan 500mg	1.488	0.77242±0.0077	140±54.72+29.088±7.074[X]	0.9913 98.27%	500±4.98	4.80	4.91	4.91	4.91	4.80	480±5.43	96%
			0.282±0.053+0.225±0.032[X]	0.9971 99.42%						0.97	485±9.78	97%

y^= Estimated response . (mV) for (n=3), [x] = ciprofloxacin conc. (mmol.L^{-1}), r= correlation coefficient, r^2% = linearity percentage,
FI – T&N= CFIA via turbidity (T_{0-180}) and scattered light at two opposite position ($2N\pm_{90}{}^{o}$) method , uv-vis sp. = uv- vis spectrophotometry method.

Paired t-test was used in order to compare the new FI–T&N method with the uv-vis-spectrophotometric (official) method. The obtained results as shown in table no.9 indicate clearly that there was no significant differences between newly FIA – T&N method and the uv- vis spectrophotometric method at 95% confidence interval as the calculated t-value is less than critical tabulated t-value.

Table no. 8: Paired t-test for the new adopted method (depend on $T_{(0-180)}$) and the uv-vis spectrophotometric method for the determination of ciprofloxacin in pharmaceutical drugs.

No. Sample	pharmaceutical drug company And Claimed content of active ingredient	Practical Content(mg)		d (mg) FI– T&N with uv-vis sp	$\bar{X}_d$ FI– T&N with uv-vis sp	σ_{n-1} FI– T&N with uv-vis sp	σ_{n-1} FI– T&N	σ_{n-1} uv-vis sp	$(X-\mu)\sqrt{n}/s$ FI– T&N with Quoted	$(X-\mu)\sqrt{n}/s$ uv-vis sp with Quoted	Paired t-test $\bar{X}_d \sqrt{n}/\sigma_{n-1}$ FI– T&N with uv-vis sp	t_{tab} at 95% confidence interval, n-1
		Proposed method FI – T&N	uv-vis sp method									
1	Ciprofloxacin BRISTOL U.K 500g	482±5.45	495±7.35	13	9	4	9.42	2.89	3.31	2.99	3.897< 4.303	
2	Ciprofloxacin USP BAL-PHARAMA LIMITED India 500mg	481±5.44	490±9.78	9			9.53	5.08	3.45	3.41		
3	Cipropharm Phama-international Jordan 500mg	480±5.43	485±9.78	5			12.64	6.28	2.74	4.14		

FI– T&N= CFIA via turbidity (T_{0-180}) and scattered light at two opposite position ($2N\pm_{90}^{o}$) method,
uv-vis sp. = uv-vis spectrophotometry method

Conclusion

The proposed FIA method is simple, rapid and it is sensitivite for the determination of ciprofloxacin – HCl in pure and pharmaceutical preparation. It was shown that with no doubt that the newly developed method is as good as the official recommended method. An alternative analytical methods is found through this research work which was based on simple parameter conditions.

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