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New Approach for the determination of ciprofloxacin hydrochloride using fluorescence resonance energy transfer (FRET) and continuous flow injection analysis via ISNAG-fluorimeter

Nagam S. Turkie[1], Hayder Q. Munshid[2*]
[1]*Department of Chemistry, College of Science, University of Baghdad, Baghdad, Iraq*
[2]*Department of Pharmacy, Al-esraa University Collage, Baghdad, Iraq*

Abstract

A new approach was used for the determination of ciprofloxacin hydrochloride (CIP-HCl) via the fluorescence resonance energy transfer (FRET) from erythrosine B (Erth-B) which used as a carrier stream. The method was applied using flow injection system of a new homemade ISNAG fluorimeter with fluorescence measurements at ± 90◦ via 2×4 solar cell. The linear range for the new developed methodology was 0.01 – 0.4 mmol/L with correlation coefficient r = 0.9653. While the L.O.D was 1.736 µg/sample from the stepwise dilution for the minimum concentration in the linear dynamic ranged of the calibration graph. The method was successfully applied to the determination of ciprofloxacin hydrochloride (CIP-HCl) in three different pharmaceutical drugs. A comparison was made between the newly developed method analysis and the classical method using t-test. It was noticed that there was no significant difference between the two methods at 95 % confidence level.

Keywords- Ciprofloxacin hydrochloride, Erythrosine B, Flow injection analysis, Fluorescence Resonance Energy Transfer (FRET).

INTRODUCTION

Ciprofloxacin hydrochloride (CIP-HCl) is a synthetic and broad-spectrum fluoroquinolone antibacterial agent structurally related to nalidixic acid, which is has the nomenclature 1-Cyclopropyl-6-fluoro-4-oxo-7-(piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid hydrochloride (Fig. 1), its empirical formula ($C_{17}H_{18}FN_3O_3$.HCl). Ciprofloxacin hydrochloride is a pale yellow, crystalline, slightly hygroscopic powder, which is soluble in water, slightly soluble in methanol, very slightly soluble in anhydrous ethanol, practically insoluble in acetone, in ethyl acetate and in methylene chloride [1, 2]. Ciprofloxacin is used to treat different types of bacterial infections, i.e.; bone and joint infections, intra-abdominal infections, certain type of infectious diarrhea, respiratory tract infections, skin infections, typhoid fever, and urinary tract infections [3]. There are several analytical methods have been reported in literatures for the determination of ciprofloxacin hydrochloride in its different forms and preparations, some of these methods are spectrophotometric methods [4-7], fluorescence [8], electrochemical [9-12], chromatographic [13-16], and flow injection analysis (FIA) methods [17, 18].

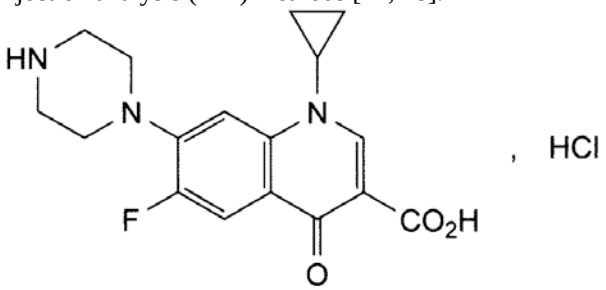


Figure1: Chemical structures of Ciprofloxacin hydrochloride.

The aim of this study was to develop a new method for the determination of ciprofloxacin hydrochloride (CIP-HCl) via the fluorescence resonance energy transfer (FRET) from erythrosine B which used as a carrier stream using the new homemade ISNAG-fluorimeter. Erythrosin B; tetra-iodofluorescein, also known as Spiro[isobenzofuran-1(3H),90-[9H] xanthen]-3-one, 30,60-dihydroxy-20,40,50,70-tetraiodo-, sodium salt. Its molecular formula $C_{20}H_6I_4Na_2O_5$ and molecular weight of 876.86 g/mol (Fig. 2), red to brown powder that's soluble in water and ethanol [19]. It is an organoiodine compound and it is cherry-pink synthetic, primarily used for food coloring [20], Erythrosin B probe is used for the molecular mobility of water-soluble and membrane-bound proteins [21].

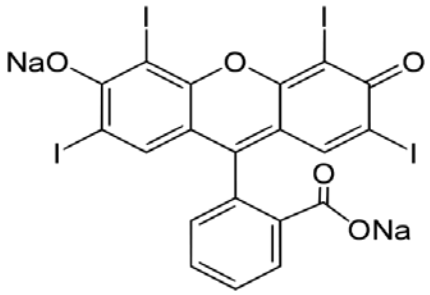


Figure2: Chemical structure of erythrosine B.

MATERIALS AND METHODS

Apparatus and Reagents

A homemade ISNAG fluorimeter [22] was used with 4-channels peristaltic pump (Ismatec, Switzerland) and Six-port medium pressure injection valve (I D E X corporation, USA) with sample loop (1 mm i.d. Teflon, variable length). Potentiometric recorder to estimate the output signals (Siemens, Germany (1- 5 V)). Spectrophotometer (UV-1800, shimadzu, Japan) was also used for classical spectrofluorometric methods.

All chemicals were used of analytical-reagent and distilled water was use to prepare all the solutions. A standard solution of 1 mmol/L of CIP-HCl and Erth-B, molecular weight 867.805 and 879.86 g/mole respectively, were prepared by dissolving 0.4339 g and 0.4399 g of CIP-HCl and Erth-B in 500 mL of distilled water. A pH range of 2.2–

8.0 buffers were prepared according to McIlvaine citric acid–phosphate buffer systems [23].

Sample preparation

Twenty tablets of three different kinds of pharmaceuticals drugs (Cipropharm Pharma International Jordan, Ciprofloxacin Bristol United Kingdom, Bactiflox Acino Switzerland) were weight and then crushed and grinded. A solution of 0.1 mmol/L were prepared by weighting 0.0066, 0.0067 and 0.0066 g (equivalent to 0.0034 g of active ingredient) from Cipropharm Pharma, Ciprofloxacin and Bactiflox respectively. Each one from these kinds of sample dissolved in distilled water. The solution was filtered to get rid of undissolved materials, the residue was washed with distilled water and completed the volume to 50 ml with the same solvent (distilled water).

Methodology

Erythrosine B (fluorescent molecule) was injected on distilled water which was used as a carrier stream through a single line manifold design using ISNAG-fluorimeter in addition to CIP-HCl treated as the same. It was noticed that the use of Erth-B gave a negative response (Fig. 3-A) which indicate the absorbance of light from LP-mercury lamp of ISNAG-fluorimeter (Fig. 4), but the solar cells cannot detect the fluorescent light because it may be not within its spectral range (i.e.; 410-1150 nm), also the use of CIP-HCl didn't gave a suitable response which could be used for the determination of this molecule (Fig. 3-B). It probably might be attributed that in case of using CIP-HCl that fluorescence might occur at the lower limit of detector response i.e.: 410 nm or little bit more. So, a new approach was used for the determination of ciprofloxacin hydrochloride via the fluorescence resonance energy transfer (FRET) from Erth-B which used as a carrier stream (Fig. 3-C). Scheme 1 shows the possible mechanism.

RESULTS AND DISCUSSION

Study of the optimum parameters

Variation of erythrosine B concentration

A series of Erth-B concentrations (5×10^{-3} – 1 mmol/L) were used as a carrier stream at a flowrate of 2.2 mL/min and 250 µL of sample segment (0.2 mmol/L of CIP-HCl) were injected. An increase in responses of the fluorescence resonance energy transfer (FRET) depend on the concentration of Erth-B up to 1 mmol/L tried as shown in fig.4. The selected of highest concentration of Erth-B molecule as it is regarded an inner irradiation source for drugs molecules and stimulate fluorescence. In order to enhance and smoothing the obtained response signal obtained from ISNAG-fluorimeter, a set (0.1632-3.774 sec. time constant) of RC-low band pass electronic filter were used. It was noticed that 0.3169 sec. (any response below this number ((time constant)) will not be measured which is in this case a fraction of seconds) is the most appropriate electronic filter for both molecules (Fig. 5).

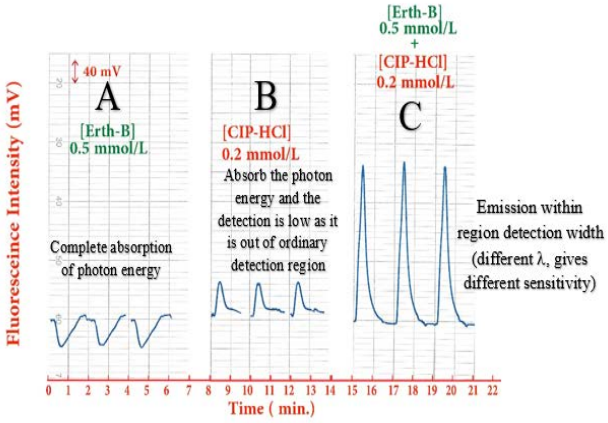
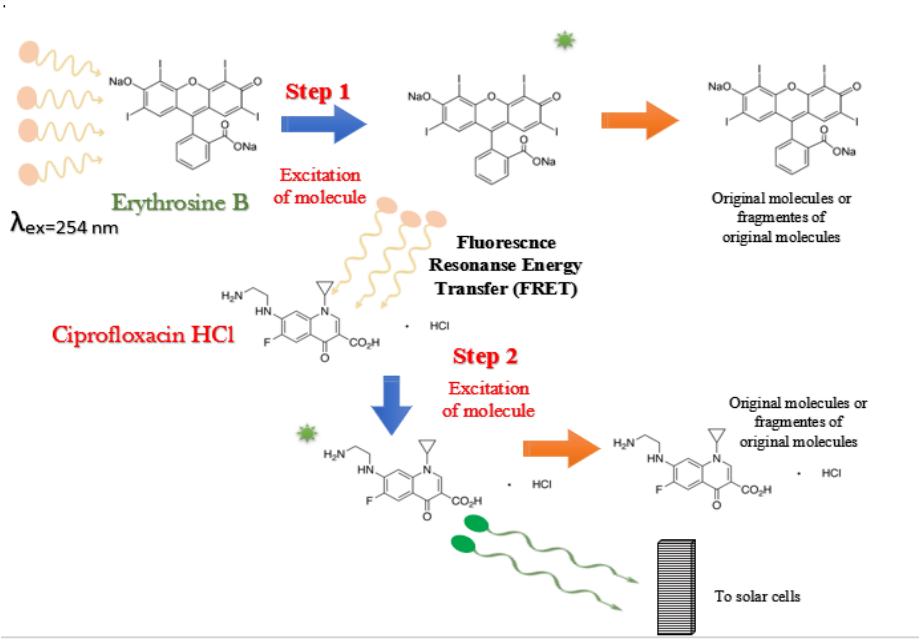


Figure3: Response profile for the fluorescence intensity of Erth-B and CIP-HCl using ISNAG-fluorimeter and 0.5 mmol/L of Erth-B.



Scheme 1: Proposed mechanism for the fluorescence resonance energy transfer (FRET).

Effect of pH on fluorescence resonance energy transfer

A series of buffer solutions were used to prepare 1 mmol/L of Erth-B which was used as a carrier stream with a flowrate of 2.2 mL/min. and a sample of 250 µL of CIP-HCl was injected in an open valve mode. It was noticed (Fig. 6-A) that the use of buffers causes a decrease in the response and followed by a slight increase and gave a minimum response at pH 8 (Fig. 6-B). An increase in pH might probably leads to the precipitation of fluorophore acceptor molecule from the energy released from Erth-B molecules or quenching the inner fluorescence in the form of non-radiative thermal energy or internal convention between electronic levels of all fluorescent molecules and losing fluorescence energy. The Erth-B prepared in distilled water gave the best response for both molecules, so it was used as the optimum for this study.

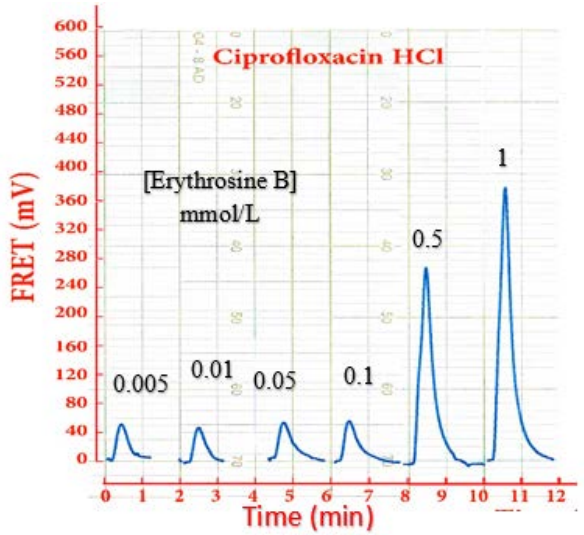


Figure 4: Variation of Erth-B concentration effect on: A: response profile-time using of 0.3169 sec. as a time constant RC-low band electronic filter.

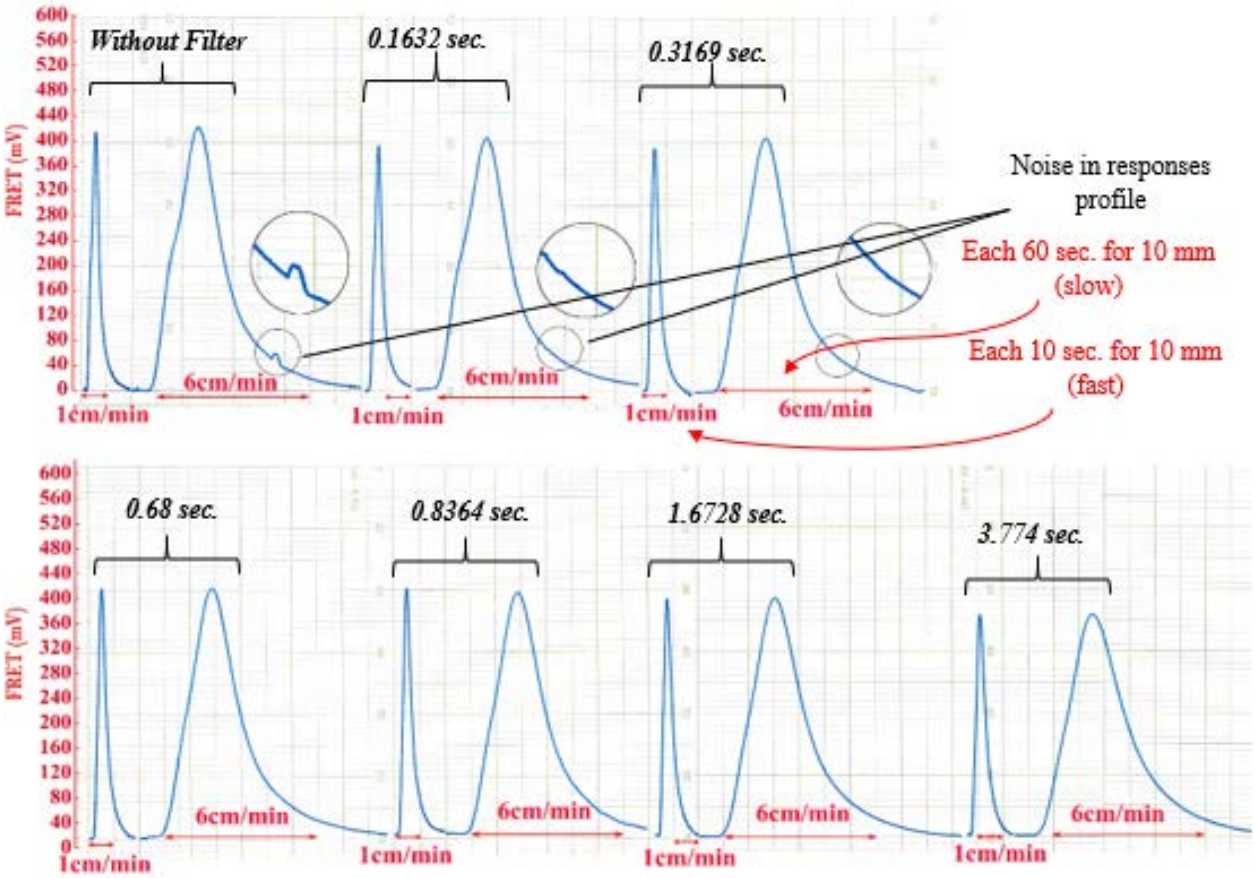


Figure 5: Response profile versus variable electronic filters using CIP-HCl.

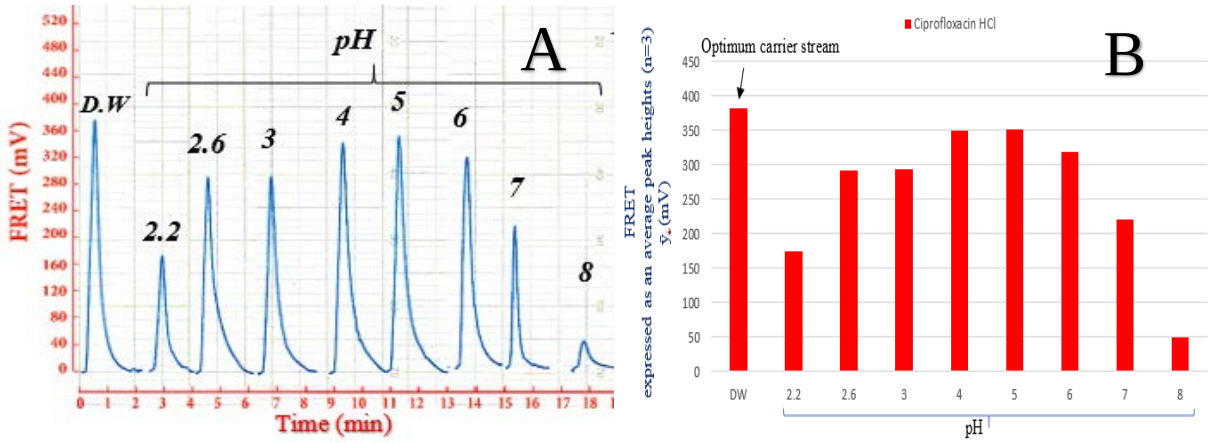


Figure 6: A; Effect of variable buffers on the response profile, B; Variation of buffers solutions on the fluorescence resonance energy transfer of 0.2 mmol/L of CIP-HCl at flowrate of 2.2 mL/min and 250 µL sample volume.

Physical parameters optimization

Effect of Flow rate

Using Erth-B prepared in distilled water as a carrier stream and 250 µL of 0.2 mmol/L of CIP-HCl as an injected sample volume. The flowrate effect was studied (0.575-4.3 mL/min.); it was noticed (Fig. 7-A) that there is a decrease in responses with increasing the flowrates and a decrease in peak base width, 1.7 mL/min was chosen as the optimum flowrate (Fig. 7-B) due to obtain high responses with sharp profile and less Δt_b; all this might be attributed to the convection effect of moving sample segment that increases and decrease of diffusion and dilution at optimum flowrates. The results obtained were summarized in table 1.

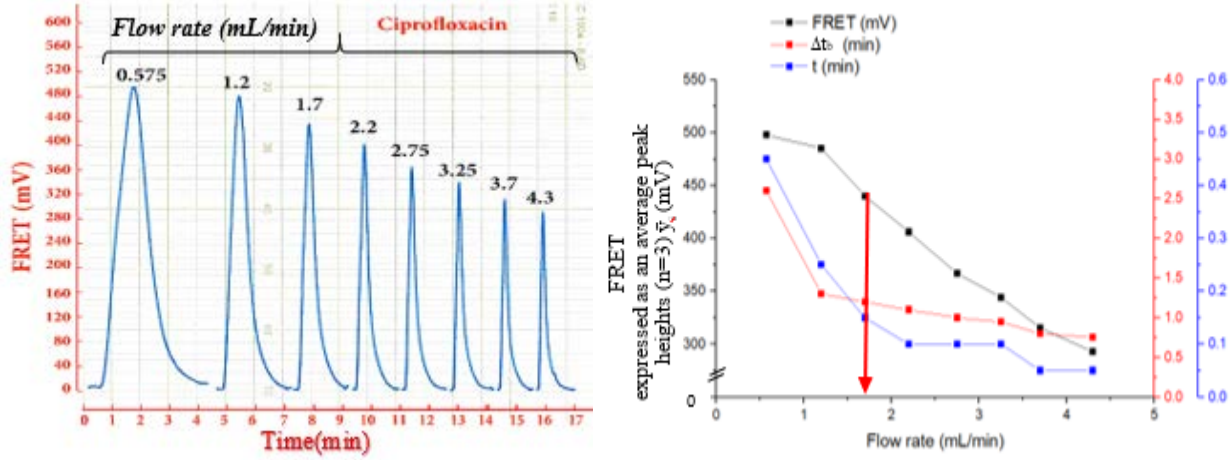


Figure 7: A; Response profile of flowrate variation using 250 µL of 0.2 mmol/L of CIP-HCl as an injected sample and Erth-B as a carrier stream., B; Flowrate variation on fluorescence resonance energy transfer, leaving time from injection valve to the measuring cell and peak base width for CIP-HCl.

Table 1: Data summery of flowrate effect study using 250 µL of 0.2 mmol/L CIP-HCl using open valve mode.

Speed of peristaltic pump (indication approximate)	Flow rate (mL/min)	Fluorescence resonance energy transfer (FRET) expressed as an average peak heights (n=3) ȳi (mV)	RSD %	Confidence interval of the average response (at 95% confidence level) $\bar{y}_i \pm t_{0.05/2,\, n-1}\, \sigma_{n-1}/\sqrt{n}$	Δt_b Peak base width (min)	t* (min)	V_{final} × (mL)	C_{final} × (mmol/L)	$Df = \frac{C_0}{C_{final}}$
5	0.575	498	0.16	498 ± 2.0372	2.6	0.45	1.745	0.0287	6.9686
10	1.200	485	0.18	485 ± 2.1117	1.3	0.25	1.810	0.0276	7.2464
15	1.700	440	0.18	440 ± 1.9626	1.2	0.15	2.290	0.0218	9.1743
20	2.200	406	0.28	406 ± 2.7825	1.1	0.1	2.670	0.0187	10.6952
25	2.750	367	0.25	367 ± 2.2856	1.0	0.1	3.000	0.0167	11.976
30	3.250	344	0.36	344 ± 3.0557	0.95	0.1	3.338	0.0150	13.3333
35	3.700	315	0.35	315 ± 2.7079	0.8	0.05	3.210	0.0156	12.8205
40	4.300	293	0.44	293 ± 3.2048	0.75	0.05	3.475	0.0144	13.8889

Effect of Sample volume

This study was carried out for the optimization of sample segment using flowrate 1.7 mL/min and different volumes of sample loop (i.e.; 50-250 µL) were used. It was noticed from fig. 8 that an increase of fluorescence intensity related with the increase of sample segment as well as increase in the peak width. Therefore, 250 µL was chosen as the best injected volume. This is due to the increased accepter molecules species of fluorescence light that is released from Erth-B (as an inner source of irradiation) and as a consequence increased fluorescence light that is emitted from drugs molecules. The released light is captured via the detector cells.

Purge time effect

Purge time effect was made using variable purge time (2-25 sec. in addition to open valve mode) by injecting 250 µL of 0.2 mmol/L of CIP-HCl as a sample segment. It was noticed, there is an increased in response profile with increasing allowed permissible time (purge time). An open valve mode was chosen as an optimum due to the stability of response profile and there is no significant difference in sensitivity in case of using 15 and 20 second with open valve mode (i.e.; 25 sec) which means completely departure of sample segment from sample loop in load position until full complete response obtained. All the data tabulated in table 2.

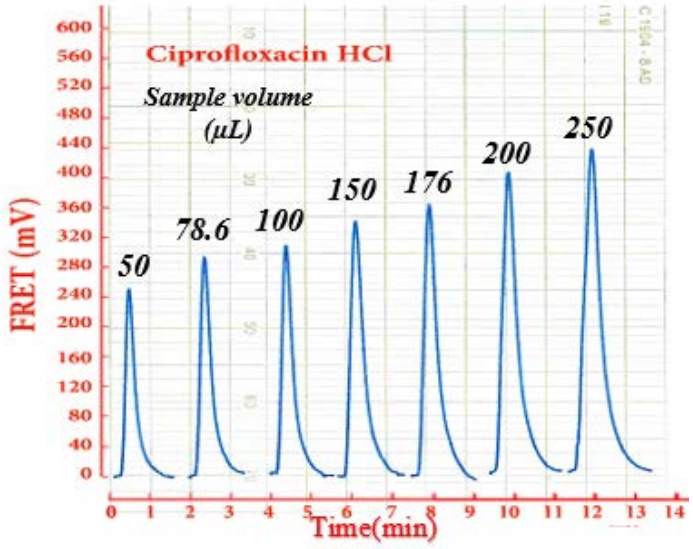


Figure 8: Effect of variation of sample volume on the fluorescence resonance energy transfer (FRET) profile of CIP-HCl.

Table 2: Effect of purge time study on FRET using ISNAG-fluorimeter.

Purge time (sec.)	Fluorescence Intensity expressed as an average peak heights (n=3) ȳi (mV)	RSD%	Confidence interval of the average response (at 95% confidence level) $\bar{y}_i \pm t_{0.05/2,\, n-1}\, \sigma_{n-1}/\sqrt{n}$
2	130	0.92	130 ± 2.9812
5	218	0.60	218 ± 3.2296
10	304	0.41	304 ± 3.0806
15	392	0.28	392 ± 2.7328
20	439	0.24	439 ± 2.6086
25 (open valve)	443	0.18	443 ± 1.9378

*open valve: Injection valve in the open mode (injection mode) until departure of fluorescence species from measuring cell.

Calibration graph for the variation of fluorescence resonance energy transfer (FRET) versus CIP-HCl

Using all the optimum parameters that achieved in previous sections; a series of CIP-HCl (0.01-1 mmol/L) were prepared in distilled water and injected on a carrier stream of Erth-B at flowrate of 1.7 mL/min, the variation of this drug concentration with the fluorescence resonance energy transfer FRET in mV obtained by ISNAG-fluorimeter. A calibration graph was plotted with a linear range of 0.01-0.4 mmol/L (Fig.9-A). In which an increase of FRET due to the increased of acceptor molecules species i.e.; CIP-HCl that is absorbed the fluorescence light released from Erth-B (as inner source of irradiation) up to 0.4 mmol/L. A comparison was made with the classical spectrophotometric method through the measurements of absorbance for each drug at different concentrations a. A calibration graph was plotted using λ_{max}= 276 nm (Fig. 9-B). All the data for the scatter plots for these two methods for both drugs were listed in table no. 3. The repeatability of efficiency of homemade ISNAG-fluorimeter was studied at fix concentration (0.2 mmol/L) at optimum parameters. The repeated measurements for eight successive injections were measured and obtained results were tabulated in table 3 which shows that the RSD% less than 0.7%. The limit of detection was studied at three different approaches i.e.; gradual dilution of lowest concentration in the calibration graph or based on the value of slope and from the linear regression plot. Table 3 summed up all this calculation value of detection limit.

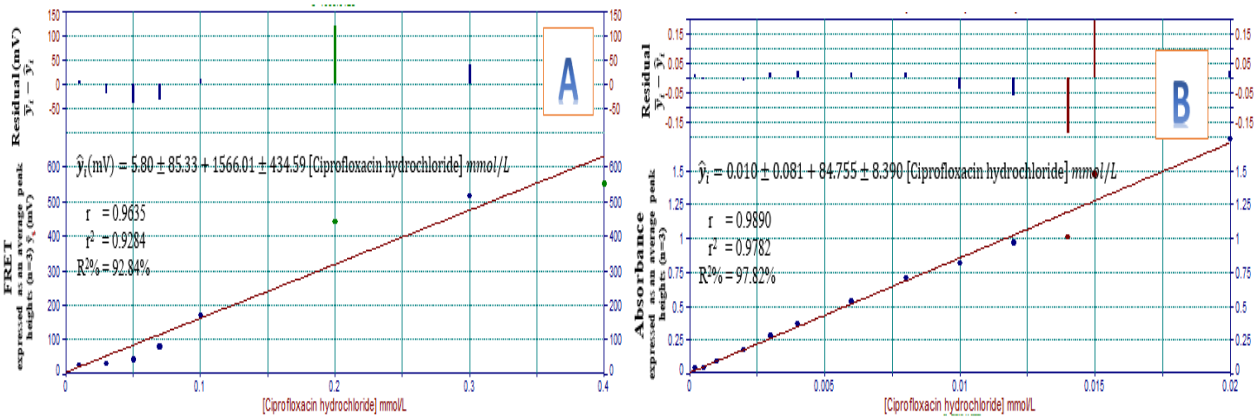


Figure 9: Linear calibration graph for CIP-HCl for; A- Newly developed methodology B- Classical method at λmax=276 nm

Table 3: Summary of calibration graph results for determination of CIP-HCl using newly developed methodology (ISNAG-fluorimeter) and classical methods at 95% confidence level.

Type of method	Range of calibration graph (mmol/L)	Equation of calibration graph $\hat{y}_i = a \pm S_a t + b \pm S_b t$ [X] mmol/L at confidence level 95%, n-2	r r^2 R^2 %	$t_{tab} = t_{0.025}$, n-2	$t_{cal} = \frac{\lvert r\rvert\sqrt{n-2}}{\sqrt{1-r^2}}$
Newly developed methodology	0.01 – 0.4 (n=8)	$\hat{y}_i$(mV) = 5.80 ± 85.33 + 1566.01 ± 434.59 [Ciprofloxacin hydrochloride] $mmol/L$	0.9635 0.9284 92.84	2.447<<8.818	
Classical method λex = 276 nm	0.002 – 0.02 (n=13)	$\hat{y}_i = 0.010 \pm 0.081 + 84.755 \pm 8.390$ [Ciprofloxacin hydrochloride] $mmol/L$	0.9890 0.9782 97.82	2.201<<22.232	

Detection limit			
[Drug minimum concentration] mmol/L	Practical based on the gradual dilution for the minimum concentration	Theoretical based on the value of slope X=3S_B/slope	Theoretical (linear equation) based on the value of $\hat{Y} = Y_b + 3S_b$
0.008	6.942×10^{-3} g/L 1.736 µg/sample	9.975×10^{-4} g/L 0.249 µg/sample	0.111 g/L 27.638 µg/sample

Repeatability			
[Drug concentration] mmol/L	Fluorescence Resonance energy transfer (FRET) expressed as an average peak heights (n=8) $\bar{y}i$ (mV)	RSD %	Confidence interval of the average response (at 95% confidence level) $\bar{y}_i \pm t_{0.05/2,\ n-1}\ \sigma_{n-1}/\sqrt{n}$
0.2	442.75	0.68	442.75 ± 4.1125

[X]: Concentration of ciprofloxacin hydrochloride or mebeverine hydrochloride in mmol/L, n: No. of measurements in calibration graph, r: correlation coefficient, r^2: coefficient of determination, R^2% (Percentage capital R Squared): explained variation as a percentage total variation. t_{tab} : $t_{0.025,11}$ = 2.201, $t_{0.025,9}$ = 2.262, $t_{0.025,8}$ = 2.306, $t_{0.025,6}$ = 2.447, X: value of L.O.D based on slope, S_B: Standard deviation of blank repeated for 13 times,Y_b: average response for blank = intercept (a), S_b: standard deviation equal to $S_{y/x}$ (residual). t_{tab}: $t_{\frac{0.05}{2},7} = 2.365$ (n = 8) two tailed.

Application of the use of ISNAG-fluorimeter for the determination of CIP-HCl in the pharmaceutical drugs

The newly developed methodology was used for the determination of CIP-HCl in three different pharmaceutical drugs from different companies (Cipropharm Pharma International Jordan, Ciprofloxacin Bristol United Kingdom, Bactiflox Acino Switzerland). The standard addition method was applied by preparing a series of solutions from each pharmaceutical drug via transferring 5 mL of each sample (0.1 mmol/L) to five volumetric flasks (10 mL), followed by the addition of (0, 1, 2, 3 and 4 mL) from 0.5 mmol/L standard solution in order to have the concentration range from 0 – 0.2 mmol/L. Fig. 10 shows the response profile. The comparison of the obtained results was made with classical spectrophotometric method at λ_{max}=276 nm. Standard addition method was applied using a series of solutions via transferring 5 mL of CIP-HCl sample (0.005 mmol/L which prepared by transferring 2.5 mL of 0.1 mmol/L stock sample to 50 mL volumetric flask) to five volumetric flasks (10 mL), followed by the addition of (0, 1, 2, 3 and 4 mL) from 0.04 mmol/L standard solution to have the range of concentration from 0 – 0.016 mmol/L. Table no. 4 explain the synopsis of standard addition graphs measurements. Values of $\%R^2$ (which shows that all explained results from out of total values); in addition to practically values in term of concentration. The results were mathematically treated [24, 25] and tabulated in table 4 at confidence level of 95%.

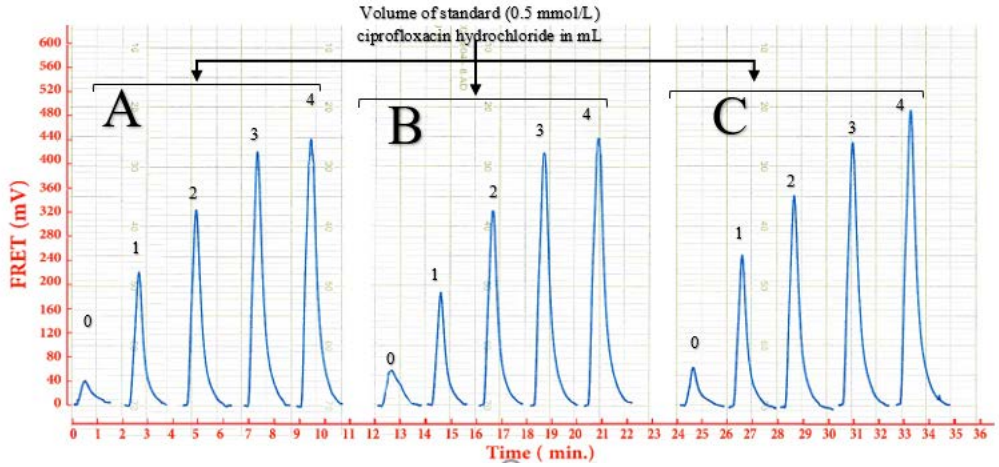


Figure 9: Response profile of standard addition method using developed method for the determination of CIP-HCl manufactured by different companies A- Cipropharm, Pharma International Jordan, B- Ciprofloxacin, Bristol United Kingdom, C- Bactiflox Acino Switzerland.

Table 4: Standard addition results for the determination of CIP-HCl in three different pharmaceuticals drugs using two methods.

Commercial name, Company, Content, Country	Newly developed methodology using ISNAG-fluorimeter (mV) UV- spectrophotometric classical method absorbance measurement at λmax = 276 nm				
	Confidence interval for the average weight of tablets $\bar{W}_i \pm 1.96\,\sigma_{n-1}/\sqrt{n}$ at 95% (g) (n=20)	Weight of sample equivalent to 0.0043 g (0.1 mmol/L) of active ingredient	Theoretical content for the active ingredient $W_i \pm 1.96\,\sigma_{n-1}/\sqrt{n}$ at 95% (g)	Equation of standard addition at 95% for n-2 $\hat{y}_i = a \pm S_a t + b \pm S_b t$ [***Ciprofloxacin hydrochloride***] ***mmol/L***	r | r^2 | R^2%
Cipropharm, Pharma International, 500 mg tab., Jordan	0.7670 ± 0.0018	0.0066	0.5 ± 0.0012	$\hat{y}_i(mV) = 93.20 \pm 122.98 + 2016 \pm 1004.12$ [***ciprofloxacin hydrochloride***] ***mmol/L***	0.9651|0.9315|93.15
				$\hat{y}_i = 0.159 \pm 0.143 + 62.825 \pm 14.462$ [***ciprofloxacin hydrochloride***] ***mmol/L***	0.9922|0.9845|98.45
Ciprofloxacin, Bristol, 500 mg tab., United Kingdom	0.7759 ± 0.0053	0.0067	0.5 ± 0.0034	$\hat{y}_i(mV) = 90.20 \pm 100.61 + 1990 \pm 821.45$ [***ciprofloxacin hydrochloride***] ***mmol/L***	0.9757|0.9519|95.19
				$\hat{y}_i = 0.153 \pm 0.137 + 61.750 \pm 14.033$ [***ciprofloxacin hydrochloride***] ***mmol/L***	0.9924|0.9849|98.49
Bactiflox, Acino, 500 mg tab., Switzerland	0.7727 ± 0.0053	0.0066	0.5 ± 0.0034	$\hat{y}_i(mV) = 110.40 \pm 107.94 + 2088 \pm 881.3$ [***ciprofloxacin hydrochloride***] ***mmol/L***	0.9746|0.9499|94.99
				$\hat{y}_i = 0.157 \pm 0.130 + 62.250 \pm 13.444$ [***ciprofloxacin hydrochloride***] ***mmol/L***	0.9932|0.9864|98.64

Commercial name, Company, Content, Country	Practical concentration (mmol/L) in 10 mL *	Weight of CIP-HCl in tablet $\bar{W}_{i(mg)} \pm 4.303\,\sigma_{n-1}/\sqrt{n}$	Efficiency of determination Recovery %	t-test: Individual t-test for compared between claim & practical value $(\bar{W}_i - \mu)\sqrt{n}/\sigma_{n-1}$	t-test: Paired t –test compared between two methods	
Cipropharm, Pharma International, 500 mg tab., Jordan	0.0462	466.1977 ± 89.5517 (σ_{n-1}=36.0465)	93.24 %	|-1.624| < 4.303	$t_{cal} = \bar{X}d\sqrt{n}/\sigma_{n-1}$	t_{tab} at 95% confidence level (n-1)
	** 2.5308×10^{-3} in 10mL 0.0506 in 50mL (diluted sample)	510.5930 ± 98.2181 (σ_{n-1}=39.5349)	102.12 %			
Ciprofloxacin, Bristol, 500 mg tab., United Kingdom	0.0453	457.1163 ± 115.5505 (σ_{n-1}=46.5116)	91.42 %	|−1.597| < 4.303	$\bar{X}d$: -20.84883 σ_{n-1}: 39.91081 |-0.904| ≪ 4.303	
	** 2.4777×10^{-3} in 10mL 0.0496 in 50mL (diluted sample)	500.5000 ± 103.9956 (σ_{n-1}=41.8605)	100.10 %			
Bactiflox, Acino, 500 mg tab., Switzerland	0.0529	533.8023 ± 54.8865 (σ_{n-1}=22.0930)	106.76 %	2.650 < 4.303		
	** 2.5221×10^{-3} in 10mL 0.0504 in 50mL (diluted sample)	508.5698 ± 63.5528 (σ_{n-1}=25.5814)	101.71 %			

$\hat{y}_i$: in mV for developed method and absorbance for classical method, r: correlation coefficient, r^2: coefficient of determination, R^2%(Percentage capital R-squared): explained variation as a percentage total variation, $t_{0.025,\infty} = 1.96\ at\ 95\%$,t_{tab}: $t_{0..025,3} = 3.182$ for n=5. $\sigma_{n-1}/\sqrt{n}$ = S.E.M standard Error of the mean, $t_{\frac{0.05}{2}} = 4.303$ for n=3, μ: claim value (g). * Practical concentration (mmol/L) in 10 mL for newly developed methodology, ** In classical method the sample concentration diluted to 0.005 mmol/L before the addition by draw 2.5 mL from 0.1 mmol/L. In classical method: 5mL of 0.005 mmol/L sample, In developed method: 5mL of 0.1 mmol/L

CONCLUSION

The newly developed method was simple, sensitivities and rapid. The comparison between this work with classical spectrophotometric method via the t-test (the comparison tools) was shown that with no doubt that newly developed method (ISNAG procedure) is a good as the classical method. An alternative analytical method is found through this research work, which based on simple parameter conditions.

REFERENCES

1. Aksoy, B., I. Küçükgüzel, and S. Rollas, *Development and validation of a stability-indicating HPLC method for determination of ciprofloxacin hydrochloride and its related compounds in film-coated tablets.* Chromatographia, 2007. **66**(1): p. 57-63.
2. Cartwright, A.C., *The British pharmacopoeia, 1864 to 2014: medicines, international standards and the state*. 2016: Routledge.
3. Nayak, S., *Comparative Evaluation Study of Different Brands of Ciprofloxacin Tablets in Local Market of State Odisha.* World Journal of Pharmacy and Pharmaceutical Sciences, 2018. **7**(5): p. 1420-1434.
4. Fouad, M.M., *Novel spectrophotometric methods for simultaneous determination of ciprofloxacin hydrochloride and dexamethasone sodium phosphate in their binary mixture.* Indo Am J Pharm Res, 2015. **5**: p. 2470-80.
5. El-Kosasy, A.M., et al., *Spectrophotometric and chemometric methods for determination of imipenem, ciprofloxacin hydrochloride, dexamethasone sodium phosphate, paracetamol and cilastatin sodium in human urine.* Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 2016. **157**: p. 26-33.
6. Bhongade, B., S. Talath, and S. Dhaneshwar, *A validated method for the quantitation of ciprofloxacin hydrochloride using diffuse reflectance infrared fourier transform spectroscopy.* International Journal of Spectroscopy, 2014. **2014**.
7. Qassim, A.W., *Spectrophotometric Determination of Ciprofloxacin Hydrochloride InPharmaceutical Formulation Ciproxin.* Int J Adv Sci Tech Res, 2015. **3**: p. 135.
8. Madrakian, T., S. Maleki, and A. Afkhami, *Surface decoration of cadmium-sulfide quantum dots with 3-mercaptopropionic acid as a fluorescence probe for determination of ciprofloxacin in real samples.* Sensors and Actuators B: Chemical, 2017. **243**: p. 14-21.
9. Zhang, X.-Z., et al., *Simple and cost-effective determination of ciprofloxacin hydrochloride by electrical micro-titration.* Chinese Chemical Letters, 2017. **28**(7): p. 1406-1412.
10. Deepak, M.P. and G.P. Mamatha, *Voltammetric Studies of Ciprofloxacin Hydrochloride at Poly (L-Tyrosine)/SnO2 Nanoparticles Modified Carbon Paste Electrode.* ANALYTICAL & BIOANALYTICAL ELECTROCHEMISTRY, 2015. **7**(5): p. 523-538.
11. Riad, S.M., et al., *Ion-Selective Membrane Sensors for the Determination of Ciprofloxacin Hydrochloride in Water and Pharmaceutical Formulation.* Analytica & Bioanalytical Electrochemistry, 2014. **6**(5): p. 559-527.
12. Okan, M., E. Sari, and M. Duman, *Molecularly imprinted polymer based micromechanical cantilever sensor system for the selective determination of ciprofloxacin.* Biosensors and Bioelectronics, 2017. **88**: p. 258-264.
13. Rote, A.R. and R.B. Saudagar, *New Analytical Method Development and Validation of Ciprofloxacin and Ornidazole in Human Plasma by High Performance Thin Layer Chromatography.* Pharmaceutical Methods, 2016. **7**(2).
14. Budiarti, A., I.G. Gandjar, and A. Rohman, *Liquid Chromatography with UV detection for Simultaneous determination of ciprofloxacin and metronidazol.* Jurnal Teknologi (Sciences and Technology), 2015. **72**: p. 1-45.
15. Ronowicz, J., et al., *Simultaneous determination of ciprofloxacin hydrochloride and hydrocortisone in ear drops by high performance liquid chromatography.* Chemical Papers, 2014. **68**(7): p. 861-870.
16. Razzaq, S.N., et al., *Stability Indicating RP-HPLC Method for Simultaneous Determination of Ciprofloxacin and Dexamethasone in Binary Combination.* Journal of the Chilean Chemical Society, 2017. **62**(3): p. 3572-3577.
17. Shakir, I.M. and R.F. Hassan, *New approach for determination of ciprofloxacin by quenched fluorescence of analytically interested species using continuous flow injection laser diode fluorimeter analyser.* Iraqi Journal of Science, 2016. **57**(1C): p. 522-533.
18. Palamy, S. and W. Ruengsitagoon, *Reverse flow injection spectrophotometric determination of ciprofloxacin in pharmaceuticals using iron from soil as a green reagent.* Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 2018. **190**: p. 129-134.
19. Sabnis, R.W., *Handbook of biological dyes and stains: synthesis and industrial applications*. 2010: John Wiley & Sons.
20. Lyday, P.A., *Iodine and iodine compounds. Ullmann's Encyclopedia of Industrial Chemistry*. 2005, Wiley-VCH, Weinheim.
21. Pravinata, L.C., Y. You, and R.D. Ludescher, *Erythrosin B phosphorescence monitors molecular mobility and dynamic site heterogeneity in amorphous sucrose.* Biophysical journal, 2005. **88**(5): p. 3551-3561.
22. Shakir , I.M.A. and N.S.T. Al-Awadie *ISNAG Fluorimeter For Fluorescence Measurement At The Visible Region Of Electromagnetic Radiation Using Mercury Tube Lamp As A Source For Irradiation For Continuous Flow Analysis Using Solar Cells*. 2014: Iraq.
23. McIlvaine, T., *A buffer solution for colorimetric comparison.* J Biol Chem, 1921. **49**: p. 183–6.
24. Miller, J. and J.C. Miller, *Statistics and chemometrics for analytical chemistry*. 2018: Pearson Education.
25. Skoog, D.A., F.J. Holler, and S.R. Crouch, *Principles of instrumental analysis*. 2017: Cengage learning.