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Research Article

TURBIDIMETRIC-FLOW INJECTION ANALYSIS METHOD FOR THE DETERMINATION OF CEFOTAXIME SODIUM IN PHARMACEUTICAL DRUGS USING AYAH 6SX1-T-1D CFIA INSTRUMENT

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ABSTRACT

A newly turbidimetric-flow injection method characterized by its speed and sensitivity has been developed for the determination of Cefotaxime sodium in pharmaceutical drugs.It is based on the formation of yellowish white precipitate for the CFTS - $K_3[Fe(CN)_6]$ ion pair in aqueous medium. Turbidity was measured by Ayah 6SX1-T-1D Solar cell CFI Analyser via the attenuation of incident light from the surface precipitated particles at 0-180°. The chemical and physical parameters were studied and optimized. The calibration graph was linear in the range of 1-50 $mMol.L^{-1}$, with correlation coefficient r = 0.9997. The limit of detection 63.739 µg/sample from the step was dilution for the minimum concentration in the linear dynamic ranged of the calibration graph with RSD% lower than 1% for 9 and 20 $mMol.L^{-1}$ (n=8,6 respectively) concentration of Cefotaxime sodium. The method was successfully applied to the determination of Cefotaxime sodium in three pharmaceutical drugs. A comparison was made between the newly developed method analysis and the classical method, in addition to between three different pharmaceutical preparations (UV- spectrophotometry at wave length 260 nm) using the standard additions method via the use of F-test. It was noticed that there was no significant difference between two methods at 95% confidence level and no significant difference between three drugs.

Keywords: Cefotaxime sodium, Flow injection analysis, Turbidity.

INTRODUCTION

Cefotaxime sodium is a Sodium (6*R*,7*R*)-3-[(acetyloxy)methyl]-7-[(2*Z*)-2-(2-aminothiazol-4-yl)-2-(methoxyimino)acetyl]amino]-8-oxo-5-thia-1-azabicyclo [4.2.0] oct-2-ene-2-carboxylate, $C_{16}H_{16}N_5NaO_7S_2$,] its molecular weight is 477.447, it is white or slightly yellow powder, freely soluble in water, sparingly soluble in methanol; **Fig.1** shows the chemical structure of CFTS[1].

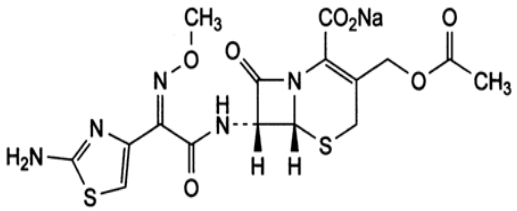


Fig. 1: following chemical structure of CFTS

Cefotaxime is considered to be broad spectrum antibiotics, primarily used to treat bacterial infections of the skin, soft tissues and the urinary tract. It belongs to an important class of antibiotics. Cefotaxime sodium is a third-generation cephalosporin antibiotic. Like other third-generation Cephalosporins, antibiotics characterized by a broad antibacterial spectrum and a resistance to beta-

lactamase-producing organisms in addition to its antimicrobial activity (streptococci, staphylococci, pneumococci, etc.)[2]. Cefotaxime failed to penetrate the central nervous system and were unsuccessful in the treatment of meningitis ,while the CFTS enter the central nervous system and reach therapeutic concentrations, there sufficient for treatment of meningitis caused by aerobic gram-negative bacteria[3], it has broad spectrum activity against Gram positive and Gram negative bacteria. In most cases, it is considered to be equivalent to ceftriaxone in terms of safety and efficacy .These characteristics are of considerable clinical and hence, analytical interest.Several analytical procedures are available in the literature for the analysis of CFTS via spectrophotometric.

Mechanism of Action

Inhibits bacterial cell wall synthesis by binding to one or more of the penicillin-binding proteins (PBPs) which in turn inhibits the final transpeptidation step of peptidoglycan synthesis in bacterial cell walls, thus inhibiting cell wall biosynthesis. Bacteria eventually lyse due to ongoing activity of cell wall autolytic enzymes (autolysins and murein hydrolases) while cell wall assembly is arrested. Cefotaxime, like other β-lactam antibiotics does not only block the division of bacteria, including cyanobacteria, but also the division of cyanelles, the photosynthetic organelles of the Glaucophytes, and the division of chloroplasts of bryophytes. In contrast, it has no effect on the plastids of the highly developed vascular plants. This is supporting the endosymbiotic theory and indicates an evolution of plastid division in land plants[5].

Clinical Use and Side Effect

CFTS is used for infections of the respiratory tract, skin, bones, joints, urogenital system, meningitis, and septicemia. It generally has good coverage against most Gram-negative bacteria, with the notable exception of Pseudomonas. It is also effective against most Gram-positive cocci except for Enterococcus. It is active against penicillin-resistant strains of Streptococcus pneumonia. It has modest activity against the anaerobic Bacteroides fragilis. To make sure CFTS is safe for you, tell your doctor if you have:

- an allergy to penicillin;
- kidney disease;
- liver disease;
- a stomach or intestinal disorder such as colitis;
- diabetes;
- a heart rhythm disorder; or
- if you also take furosemide.

This medicine is not expected to harm an unborn baby .CFTS can pass into breast milk and may harm a nursing baby[6].

EXPERIMENTAL

All chemicals were used of analytical-reagent grade and distilled water used to prepare the solutions. A standard solution (0.05 Mol.L^{-1}) of Cefotaxime sodium (CFTS) $C_{16}H_{16}N_5NaO_7S_2$ (477.447 g. mol^{-1}) was prepared by dissolving11.936 g in 500 ml distilled water. A stock solution (0.1Mol.L^{-1}) of Potassium hexacyanoferrate(III) $K_3[Fe(CN)_6]$, (329.26 g.mol^{-1}, Fluka) was prepared by dissolving 8.232g in distilled water , filter and dilute to 250 ml. A stock solution (0.1Mol.L^{-1}) of Potassium dichromate $K_2Cr_2O_7$ (294.18 g.mol^{-1} BDH) was prepared by dissolving 2.942 g in100 mL distilled water. A stock solution (0.1 Mol.L^{-1}) of Sodium nitroprusside $Na_2Fe(CN)_5NO.2H_2O$ (298.00 g.mol^{-1}, M&B) was prepared by dissolving 2.450 g in 250 mL distilled water, A stock solution (0.1Mol.L^{-1}) of Potassium Chromate K_2CrO_4 (194.20 g.mol^{-1}, Fluka) was prepared by dissolving 1.942 g in100 mL distilled water, A stock solution (0.1 Mol.L^{-1}) of Phosphotungstic acid (PTA Anhydrous) ($H_3PW_{12}O_{40}$ 2880.2 g.mol^{-1} Hopkin & Williaswas) prepared by dissolving 28.802g (Dissolved amount of PTA in few drops of phosphoric acid, followed by heating until complete dissolution. The solution is completed to the required volume in 100 mL volumetric flask), A stock solution (0.1 Mol.L^{-1}) of Phosphomolybdic acid (anhydrous) (PMA) ($H_3PMo_{12}O_{40}$ 1825.25 g.mol^{-1}, BDH) was prepared by dissolving 91.263 g in500 mL distilled water. A stock solution of acids Hydrochloric acid HCl (35% w/w,1.18 g.mL^{-1}, BDH, 1 Mol.L^{-1}) Sulfuric acid H_2SO_4 (96% w/w, 1.84 g.mL^{-1}, BDH, 1 Mol.L^{-1}) Phosphoric acid H_3PO_4 (88% w/w, 1.75 g.mL^{-1}, BDH, 1 Mol.L^{-1}). Acetic acid CH3COOH (99.5% w/w, 1.05 g.mL^{1}, BDH, 1 Mol.L^{-1}) all were prepared by pipetting 88.28 mL, 55.52 mL, 63.39 mL and 57.47 mL respectively of concentrated acids and complete the volume with distilled water to 1000 mL volumetric flasks. Each acid was standardized against standard solution from Na_2CO_3 (BDH, 105.99 g/mol, 0.1 Mol.L^{-1}); which prepared by dried in an oven at 115 ^oC for overnight before weighting. Sodium chloride NaCl (0.5 Mol.L^{-1}) was prepared by dissolving 0.146 g in 50 mL distilled water. Potassium bromide KBr (0.5 Mol.L^{-1}) was prepared by dissolving 2.975 g in 50 mL distilled water. Potassium nitrate KNO_3 (0.5

Mol.L^{-1}) was prepared by dissolving 2.525g in 50 mL distilled water. Potassium Chloride KCl(0.5 Mol.L^{-1}) was prepared by dissolving 1.363 g in 50 mL distilled water.

Sample Preparation

A batch of five vials were weighted, each of drug containing 1g of Cefotaxime sodium supplier from different manufacture (Claforan-Sanofi aventis-France), (CEFOTAXIME-*L D P*- Spain), and (CETAX-AUROBINDO-India) were weighted: 2.497, 2.354 & 2.443g respectively which is equivalent to 2.387g of active ingredient to obtain 50 mMol.L^{-1}. The powder was dissolved in distilled water, and complete the volume to 100 mL with distilled water.

Apparatus

The flow system used for the determination of CFTS is shown schematically in Figure 2, Peristaltic pump – 2 channels variables speed (Ismatec, Switzerland), Injection valve with valve 6-port medium pressure (IDEX corporation, USA) with sample loop (0.7mm i.d. Teflon, different length). The response was measured by a homemade Ayah 6SX1-T-1D Solar cell CFI Analyser , which uses a six snow-white light emitting diode LEDs for irradiation of the flow cell at 2 mm path length. One solar cell used as a detector for collecting signals via sample travel for 60 mm length. The readout of the system composed of x-t potentiometric recorder (Kompenso Graph C-1032) Siemens (Germany), this recorder measured by (1-500) mV or voltage and digital AVO-meter (auto range) (0-2volt) (China). UV spectrophotometer digital double beam type UV-1800, Shimadzu, Japan was used to scan the spectrum of CFTS using 1 cm quartz cell.

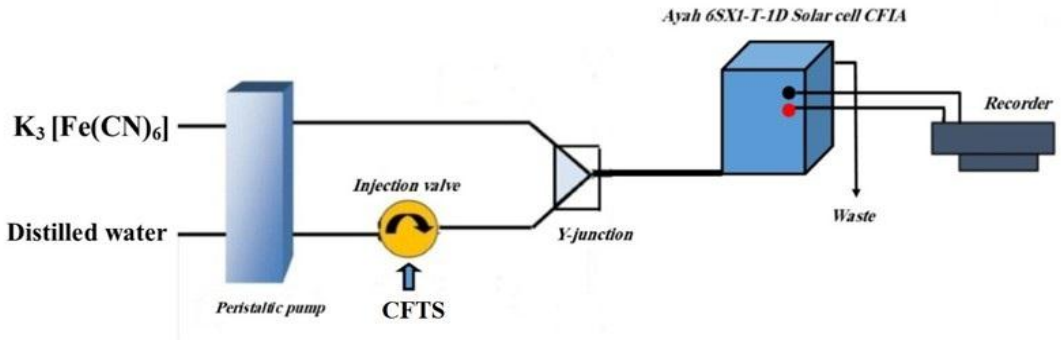
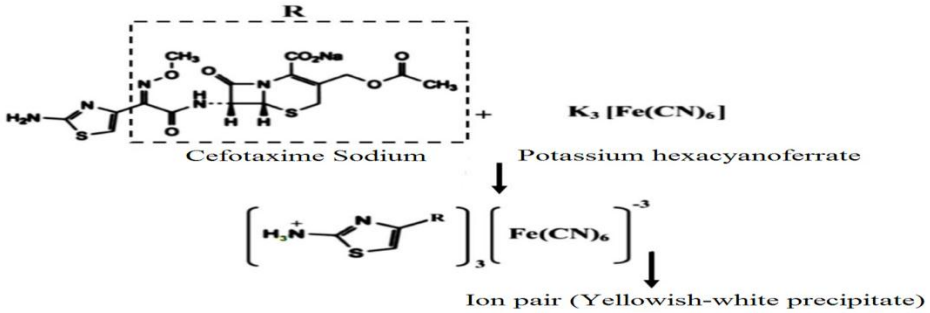


Fig. 2: Flow diagram manifold system used for the determination of CFTS

METHODOLOGY

Fig.2 shows the details of the flowgram system used for the determination of CFTS by the attenuation of incident light for the reaction of CFTS (7mMol.L^{1}) with $K_3[Fe(CN)_6]$ (50mMol.L^{-1}) in aqueous medium. A proposed expected mechanism for this reaction[7,8] as shown in the **Scheme.1.** It is composed of two lines. The first line at a flow rate of 1.3 ml.min^{-1} include distilled water passing through the injection valve to carry the sample segment (CFTS, 200 µl, at purge time 35 sec) to meet the $K_3[Fe(CN)_6]$ solution loaded by the second line at flow rate of 1ml.min^{-1} where they met at a Y-junction point before it enters to the Ayah 6SX1-T-1D Solar cell CFI Analyser. The profile was recorded when the applied voltage for the six snow white light emitting diodes (LEDs) was 1.65 volt DC for each single LED. Each injected solution was assayed in triplicate. The response profile of which was recorded on x-t potentiometric recorder to measure energy transducer response expressed as an average (n=3) peak heights in mV by attenuation of incident light.



Scheme. 1: Probable proposed mechanism of reaction between CFTS with $K_3[Fe(CN)_6]$

Optimization of Variable

Chemicals parameters (mainly effect of different reagents, concentration of reagent and type of carrier stream for the system) of CFTS with $K_3[Fe(CN)_6]$ as well as physical parameters (flow rate, sample volume, purge time, volume of reaction coil if necessary & intensity of light) were studied using two lines manifold system (**C.F. Fig.2**).

Chemical Variables

Effect of Different Types of Reagents

The study was carried out using series of solutions prepared by different reagents ($K_2Cr_2O_7$, $Na_2Fe(CN)_5NO.2H_2O$, K_2CrO_4, PTA, PMA & $K_3[Fe(CN)_6]$). Each single reagent was prepared as 0.05 $Mol.L^{-1}$ in 25 mL volumetric flasks. Injection volume was 200 µl and flow rate 1.3, 1 $mL.min^{-1}$ for the carrier stream and reagent respectively. **Fig.3 A,B** shows the variation of transducer energy response expressed as an average peak heights (n=3) in mV with different reagent**.** The obtained results were tabulated in **Table.3**, which Summarizes the average of three successive measurement with relative standard deviation and confidence interval of the average response at 95% confidence. It was noticed that $K_3[Fe(CN)_6]$ gave an increase in the attenuation of incident light (-ve response); while using the rest of reagent did not give satisfactory results. Based on the responses obtained, $K_3[Fe(CN)_6]$ was the choice to use for the assessment of CFTS. Therefore, the following paragraph will describe the effected of variation of concentration of this reagent.

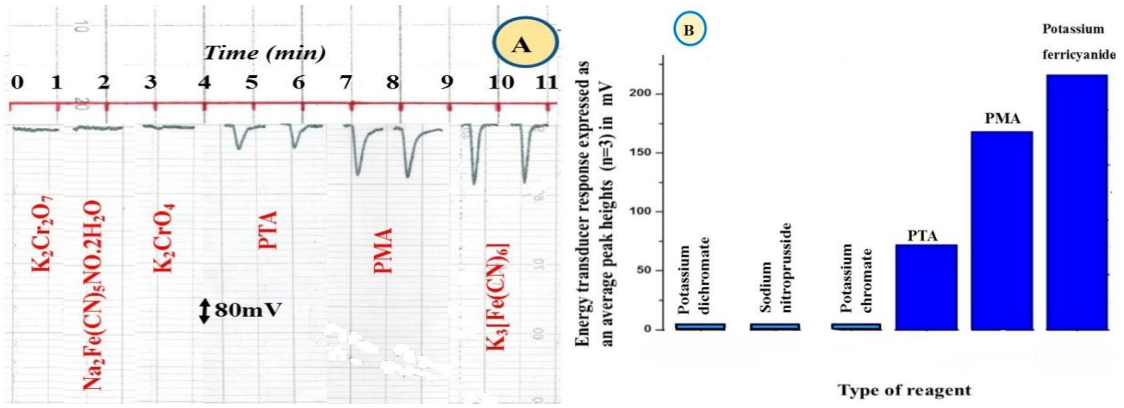


Fig. 3: Effect of different types of reagent on:
(A): Response profile versus time,
(B): Energy transducer response expressed as an average peak heights using (7mMol.L^{-1}) concentration of CFTS

Table 3: Effect of different types of reagents at fixed concentration on the transducer energy response for determination of CFTS

Type of reagent	Energy transducer response expressed as an average peak heights (n=3) $\bar{y}_i$ in (mV)	RSD%	Confidence interval at (95%) $\bar{y}_i \pm t_{0.05/2,n-1}\,\sigma_{n-1}/\sqrt{n}$
$K_2Cr_2O_7$	0	0	0
$Na_2Fe(CN)_5NO.2H_2O$	0	0	0
K_2CrO_4	0	0	0
PTA	72	1.36	72 ±2.433
PMA	168	0.77	168 ±3.214
$K_3[Fe(CN)_6]$	216	0.64	216 ±3.434

Effect of Potassium Hexacyanoferrate(III) $K_3[Fe(CN)_6]$ Concentration

Variables concentration of precipitating reagent (7-100) $mMol.L^{-1}$ were prepared. 200µl sample volume was injected through the carrier stream (distilled water). 7$mMol.L^{-1}$ concentration of CFTS was injected with 1.3 & 1 $ml.min^{-1}$ flow rate for carrier stream and reagent respectively in addition to 1.65 V applied voltage to the source (6 LEDs). **Fig. 4-A,B** shows that 70 $mMol.L^{-1}$ of $K_3[Fe(CN)_6]$ is the optimum concentration. While at higher concentration (>70 $mMol.L^{1}$) lead to decrease in the height of negative response, this might be attributed to the coagulation of solid precipitated particles ; thus increase the voids between these glommurate causing the increase of clear intensity of light. Therefore, 70$mMol.L^{-1}$ was selected as optimum concentration of $K_3[Fe(CN)_6]$.The results were summarized in **Table.4.**

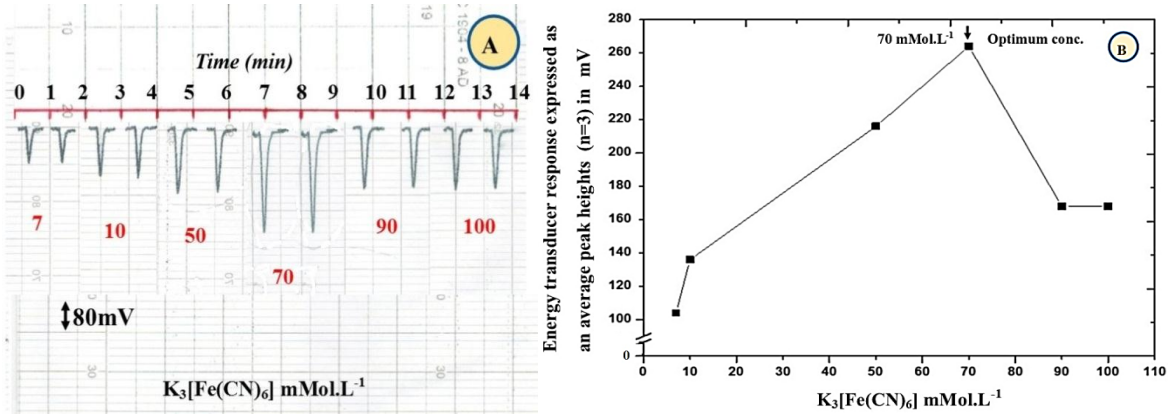


Fig. 4: Variation of $K_3[Fe(CN)_6]$ concentration
(A): response profile for CFTS-$K_3[Fe(CN)_6]$ system.
(B): transducer energy response (mV) expressed as an attenuation of incident light.

Table 4: Variation of $K_3[Fe(CN)_6]$ concentration on the transducer energy response for CFTS- $K_3[Fe(CN)_6]$ system

$K_3[Fe(CN)_6]$ mMol.L^{-1}	Energy transducer response expressed as an average peak heights (n=3) $\bar{y}_i$ in (mV)	RSD%	Confidence interval at (95%) $\bar{y}_i \pm t_{0.05/2,n-1}\,\sigma_{n-1}/\sqrt{n}$
7	104	0.98	104 ±2.532
10	136	0.79	136 ±2.669
50	216	0.48	216 ±2.576
70	264	0.34	264 ±2.230
90	168	0.29	168 ±1.210
100	168	0.35	168 ±1.461

Effect of Acidic Media

The precipitation of CFTS by $K_3[Fe(CN)_6]$ as a reagent was studied in the different acidic media (Acetic acid, Phosphoric acid, Sulfuric acid and Hydrochloric acid) at 0.1Mol.L^{-1} concentration in addition to the aqueous medium. **Fig.5 A,B** shows the variation of transducer energy response expressed as an average peak heights (n=3) in mV with different media **;**Which shows that when different acids used as a carrier streams gave in general distorted or deformed unstable profile **;** in addition to the formation of precipitates at the injection valve in which CFTS sample solution is injected an this in turn leads to closure (blocker) of injection valve **.**This might possibly due to the negative radical anion resulted from the dissociation of used acids which might lead to the precipitation of CFTS previous to the reaction with $K_3[Fe(CN)_6]$ and on this basis a return to the use of distilled water was sticked to as the most suitable carrier for the studied in hand reaction system**.** **Table.5** summed up the results which obtained from this studied**.**

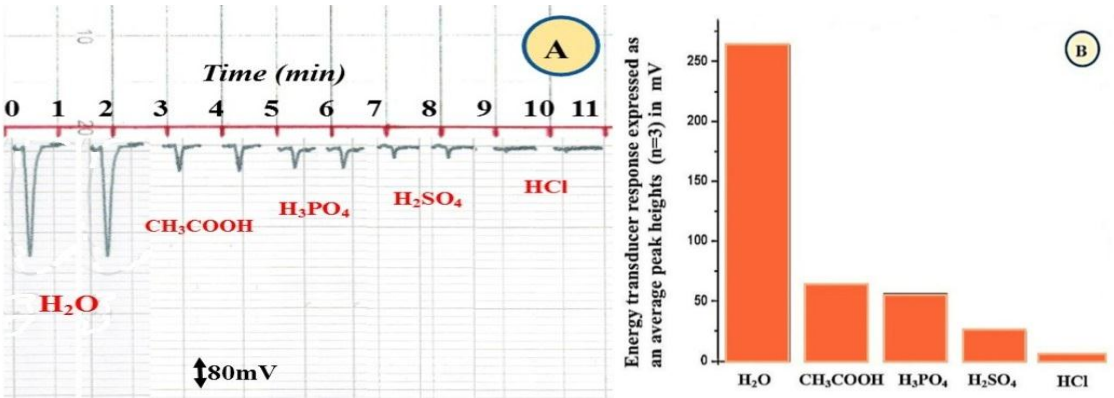


Fig. 5: Effect of different acids concentration on :
(A): Response profile versus time,
(B): Energy transducer response expressed as an average peak heights using CFTS (7 mMol.L^{-1})-$K_3[Fe(CN)_6]$ (70 mMol.L^{-1}) system

Table 5: Effect of different acids concentration as a carrier stream on the transducer energy response for determination of CFTS

Type of Medium	Energy transducer response expressed as an average peak heights(n=3) ȳi (mV)	RSD%	Confidence interval at (95%) $\bar{y}_i \pm t_{0.05/2,n-1}\,\sigma_{n-1}/\sqrt{n}$
H2O	264	0.34	264±2.230
CH_3COOH	64	1.92	64±3.053
H_3PO_4	56	2.29	56±3.186
H_2SO_4	24	5.96	24±3.554
HCl	0	0	0

Effect of Reaction Media (as a Carrier Stream) on Ion Pair Formation

The ion pair of CFTS (7 $mMol.L^{-1}$)-$K_3[Fe(CN)_6]$ (70 $mMol.L^{-1}$) system was studied in different salts medium (potassium bromide, potassium nitrate, potassium chloride & sodium chloride) at 0.25 $Mol.L^{-1}$ concentration in addition to distilled water as a carrier stream. **Fig.6 A,B** shows the plot, it can be seen that there is no significant difference among salts used .The distilled water was chosen as optimum carrier stream, because of its properties as well as being a suitable medium for best response. **Table.6** shows the obtained results.

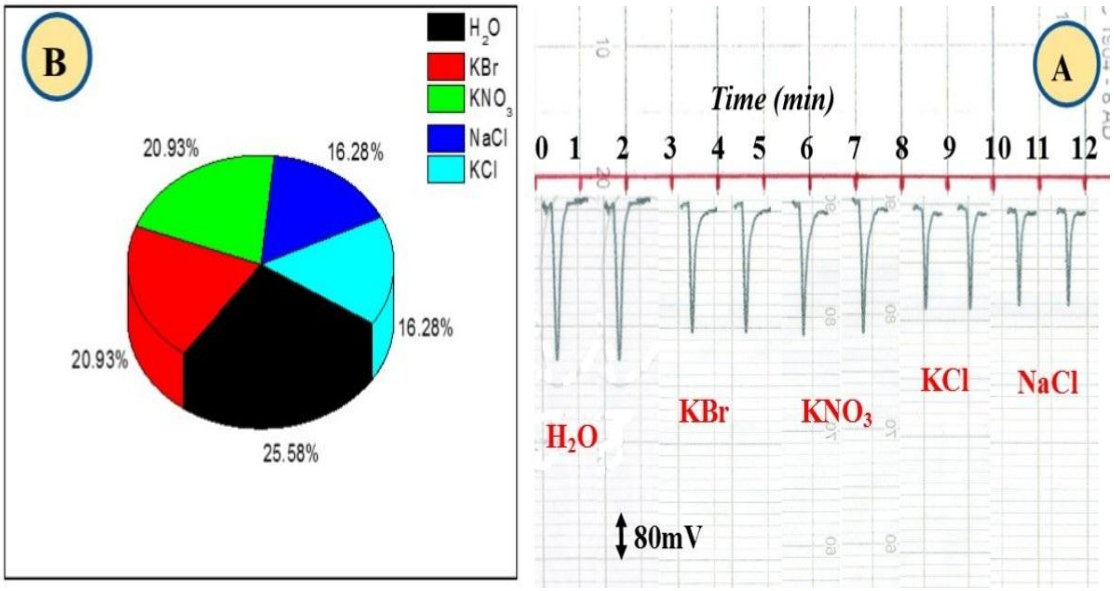


Fig. 6: Effect of the different salts medium on:
(A): Response profile versus time using 200µl sample volume
(B): Energy transducer response in mV; expressed in pie representation

Table 6: Effect of different salts on the measurement of energy transducer response for determination of CFTS using 200µl sample volume and $K_3[Fe(CN)_6]$ as an precipitating agent

Type of medium	Energy transducer response expressed as an average peak heights (n=3) ȳi (mV)	RSD%	Confidence interval at (95%) $\bar{y}_i \pm t_{0.05/2,n-1}\,\sigma_{n-1}/\sqrt{n}$
H_2O	264	0.38	264 ±2.492
KBr	216	0.50	216 ±2.683
KNO_3	216	0.64	216 ±3.434
NaCl	168	0.76	168 ±3.172
KCl	168	0.74	168 ±3.089

Physical Variables

Flow Rate

Using optimum concentration of the reactant, $K_3[Fe(CN)_6]$ 70 $mMol.L^{-1}$ and a chosen concentration of 7 $mMol.L^{-1}$ of CFTS, sample volume 200 µl with a variable range 0.15-2.6 $mL.min^{-1}$ flow rate for the carrier stream and 0.15-2 $mL.min^{-1}$ of $K_3[Fe(CN)_6]$ solution line were studied**. Fig.7 A,B** shows the

effect of flow rate on profile attenuation of incident light &Δt_B. 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 **Fig 8.A** leading to an irregular response profile. But at high flow rate influence led to an increase in peak height, decrease the peak base width, and decrease time that required for arrival the precipitate particles to the measuring flow cell up to pump speed 20 **Fig.8.B;** followed by decrease attenuation of incident light (decrease of peak height for negative response) due to increase the effect of physical parameters especially dilution effect and dispersion form convection .So, a compromise between sensitivity, peak shape, complete the reaction, and consumption of the chemicals, 1.6 &1.2 ml.min^{-1} will be used as optimum flow rate for the carrier stream and reagent respectively. The obtained results were tabulated in **Table.7**

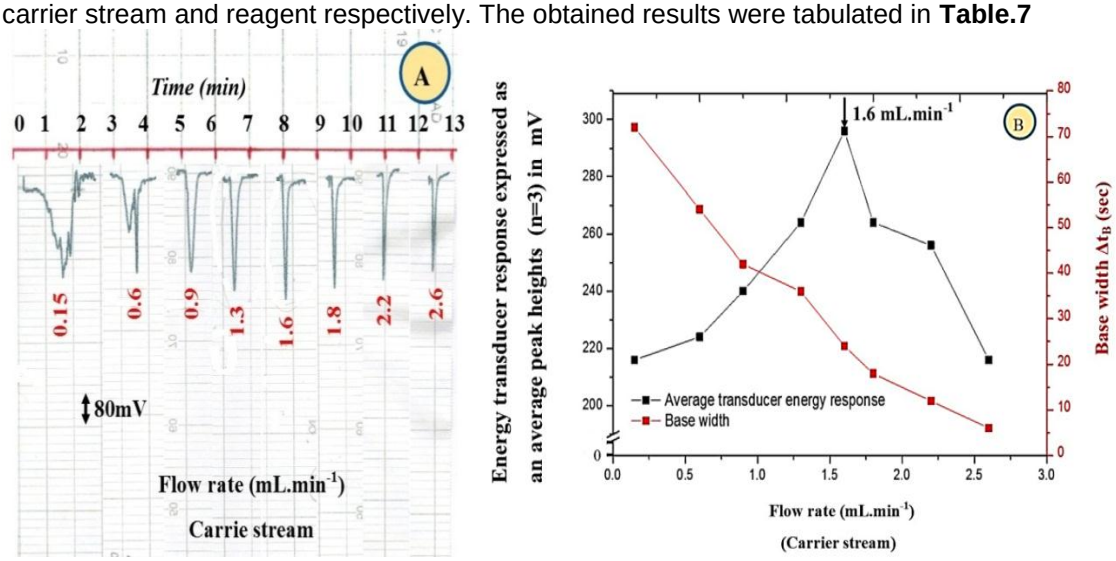


Fig. 7: variation of flow rate on:
(A): Response profile using Ayah 6SX1-T-1D solar cell CFIA, for determination of CFTS.
(B):Transducer energy response for CFTS, Peak base width (Δt_B) and time for the departure of sample segment from injection valve reaching to the measuring flow cell; using CFTS (7 mMol.L^{-1})- $K_3[Fe(CN)_6]$ (70 mMol.L^{-1}) system&35 sec purge time

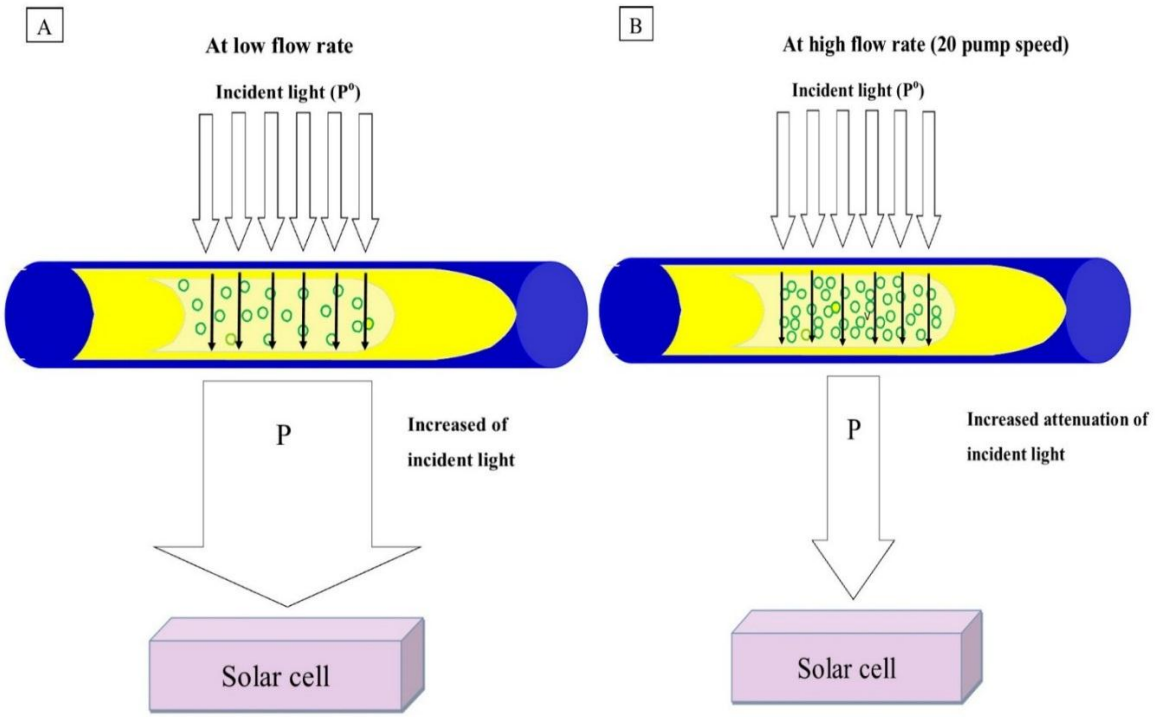


Fig. 8: General description for the effect of flow rate on distribution of precipitates particles
A: At low flow rate lead to increase of dispersion.
B: Optimum flow rate lead to decrease dilution and dispersion

Table 7: Variation of flow rate (ml.min^{-1}) on the transducer energy response mV

Pump speed (approximate)	Flow rate ml .min^{-1}		Energy transducer response expressed as an average peak heights (n=3) $\bar{y}_i$ in (mV)	RSD%	Confidence interval at (95%) $\bar{y}i \pm t0.05/2,n-1\ \sigma n-1/\sqrt{n}$	Base width Δt_B (sec)	t* sec	V* ml	C* mMol.L-1
	Carrier stream	Rea gent							
5	0.15	0.15	216	0.88	216 ±4.722	72	36	0.56	2.5
10	0.6	0.5	224	0.59	224 ±3.283	54	30	1.172	1.195
15	0.9	0.8	240	0.61	240 ±3.637	42	24	1.376	1.017
20	1.3	1	264	0.45	264 ±2.951	36	18	1.58	0.886
25	1.6	1.2	296	0.37	296 ±2.721	24	18	1.32	1.061
30	1.8	1.4	264	0.50	264 ±3.279	18	12	1.16	1.207
35	2.2	1.6	256	0.53	256 ±3.371	12	6	0.96	1.458
40	2.6	2	216	0.69	216 ±3.703	6	5.4	0.66	2.121

t* = Departure time for sample segment from injection valve to the measuring cell
V* = Volume of segment at flow cell
C* = Concentration of segment at flow cell

Sample Volume

A study was carried out for the effect of injected sample volume using the optimum parameters achieved in previous sections. The optimum concentration of $K_3[Fe(CN)_6]$ (70 mMol.L^{-1}) as precipitating reagent and a selected concentration(7 mMol.L^{-1}) of CFTS as analyst was used. Different lengths of sample loop which is equivalent to 126-290 µl successively using 35 sec for purge time. **Fig.9 A,B** It was noticed that an increase of sample volume up to 267 µl leads to a significant increase in response height & more perceptible than low sample volume as shown in **Fig.9.A,B.** While a larger sample volume i.e: more than 267 µl leading to a decrease in the attenuation of incident light might be due to continuation of the passage of carrier stream through the injection valve which in turn to cause an increase in the dispersion for the precipitated particles that cause an increase in the transmitted light . Therefore , 267 µl was chosen as an optimum sample volume to compromise between minimize the consumption of reactions solutions and sensitivity. All results was tabulated in **Table.8.**

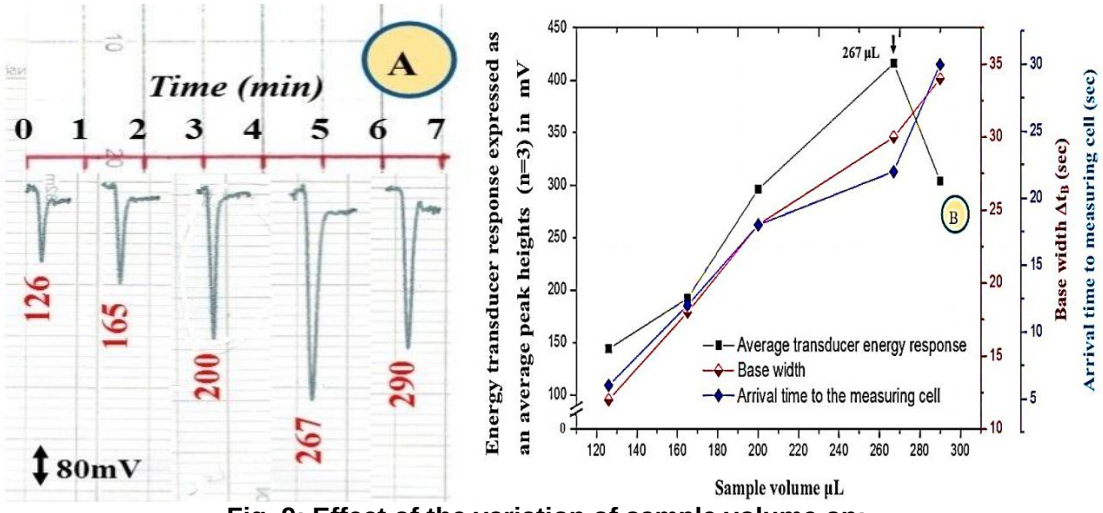


Fig. 9: Effect of the variation of sample volume on:
(A): Response profile by attenuation light using Ayah 6SX1-T-1D -Solar cell CFI Analyser versus time. (B): Energy transducer response expressed as an average peak heights in mV

Table 8: Effect of the variation of sample volume on the energy transducer response for determination of CFTS using CFTS-$K_3[Fe(CN)_6]$ system in aqueous media 35 sec as a purge time

Loop length Cm r=0.5 mm	Sample volume µL	Energy transducer response expressed as an average peak height (n=3) $\bar{y}_i$ in (mV)	RSD%	Confidence interval at (95%) $\bar{y}_i \pm t0.05/2,n-1\ \sigma n-1/\sqrt{n}$	Base width Δt_B (sec)	t* sec
16	126	144	0.78	144±2.790	12	6
21	165	192	0.63	192±3.005	18	12
25	200	296	0.45	296±3.309	24	18
34	267	416	0.26	416±2.687	30	22
37	290	304	.390	304±2.945	34	30

t* = Departure time for sample segment from injection valve to the measuring cell
$\Delta t_{B=}$ Base width of response

Purge Time of the Injected Sample

Using optimum parameter that were achieved in the previous sections, variable purge Time ((5-35sec) in addition to open valve mode) of the sample to be injected through the carrier stream was studies. **Fig.10.A** shows continuation of the increase in the attenuation of incident light expressed as peak height(negative responses) with increase of allowed permissible time up to open valve. The obtained results is tabulated in the **Table.9** and **Fig.10.B** shows a maximum response profile at open valve mode; which indicate that a completely removed of the sample segment from the sample loop in the injection valve at this time (i.e: open valve mode).

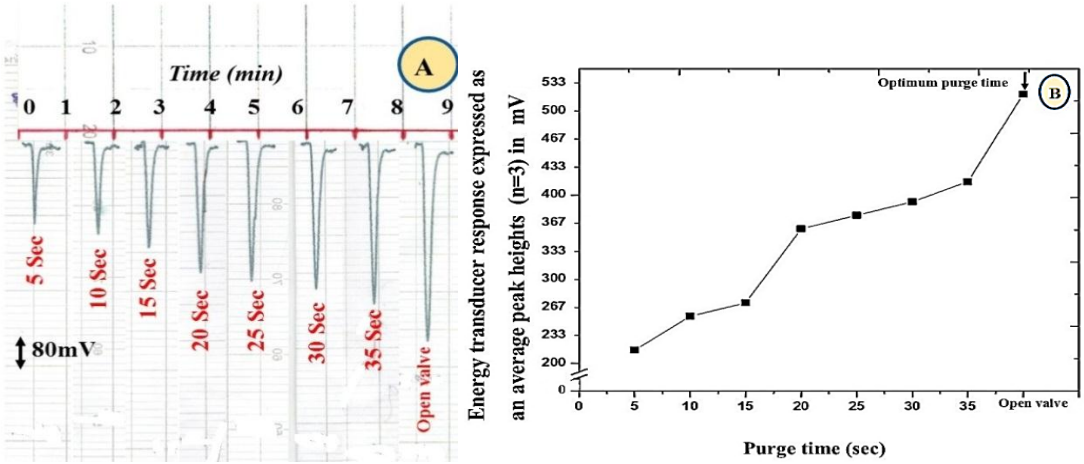


Fig. 10: Variation of purge time on:
(A): response profile using Ayah 6XS1-T-1D solar cell CFIA Analyser
(B): transducer energy response using optimum condition; using CFTS (7 mMol.L^{1})-$K_3[Fe(CN)_6]$ (70mMol.L^{-1}) system & 1.65 volt DC

Table 9: Variation of purge time on the transducer energy response using 267 µl

Purge time (sec)	Energy transducer response expressed an average peak heights (n=3)$\bar{y}_i$ (mV)	RSD %	Confidence interval at 95% $\bar{y}_i \pm t0.05/2,n-1\ \sigma n-1/\sqrt{n}$
5	216	0.77	216±4.132
10	256	0.62	256±3.943
15	272	0.57	272±3.852
20	360	0.42	360±3.756
25	376	0.39	376±3.643
30	392	0.36	392±3.506
35	416	0.32	416±3.307
Open valve	520	0.21	520±2.713

Effect of Reaction Coil Length

Using CFTS (7 mMol.L^{-1})- $K_3[Fe(CN)_6]$ (70 mMol.L^{-1}) system. The effect of reaction coil was studied .The reaction coil length has a large role in the homogenization and completion of chemical reaction. Different coil length (0-100) cm was used, this rang of lengths comprises a volume of 0-0.785 ml which connected after Y-junction directly in flow system **(Fig.2A). Fig. 11-A,B** shows that a decrease in peak height with increase coil length ,at the same time increase of the base width(ΔtB), in addition to, broadening at the peak maxima and increase of departure time for sample segment from injection valve to the measuring cell, which might probably attributed to the increase effect of the dilution and dispersion, in addition to causes the increase the accumulation and compactness of particles leading to a large particles and an increase inter particles spaces that increase the transmitted light. Therefore ; two lines manifold system without reaction coil necessary for completion of precipitate CFTS by $K_3[Fe(CN)_6]$ in aqueous medium**. Table.10** shows all results of coil effect on energy transducer response.

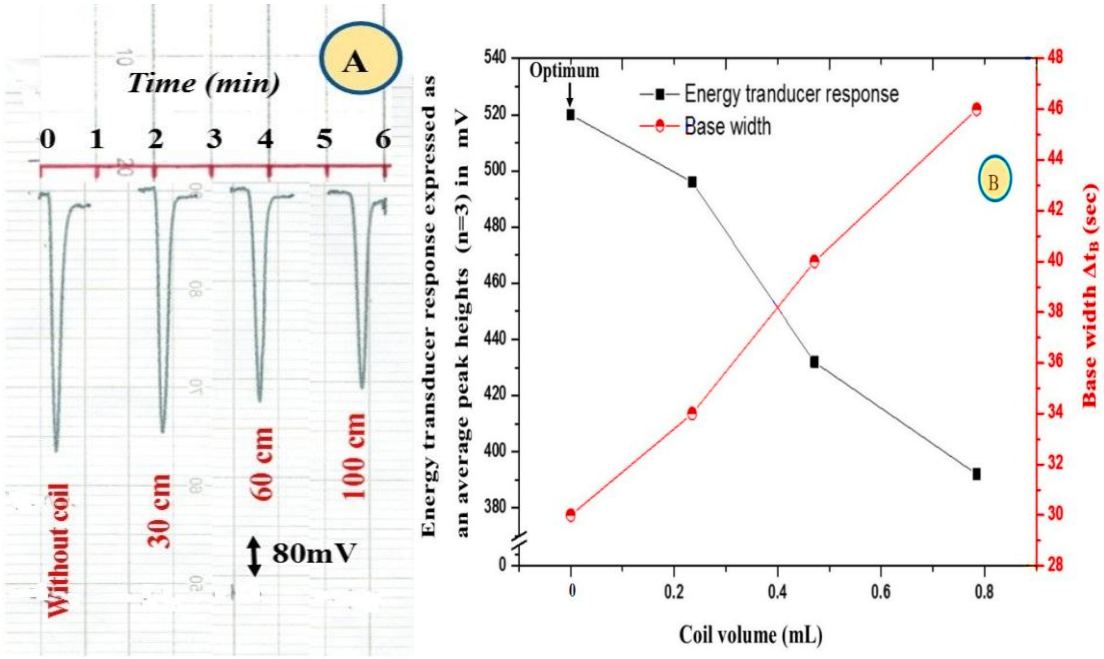


Fig.11: Effect of reaction coil on :
(A): Response profile versus time.
(B): Energy transducer response expressed as an average peak heights in mV&Δt_B using CFTS (7 mMol.L^{-1}) – $K_3[Fe(CN)_6]$ (70mMol.L^{-1}) system in aqueous medium

Table 10: Effect of coil length on energy transducer response expressed as an average peak heights mV for determination of CFTS using optimum parameters (i.e: chemical & physical variable)

Coil length (cm)	Coil volume (ml) $r^2\pi$ h, r =0.5 mm	Energy transducer response expressed as an average peak heights (n=3) $\bar{y}_i$ (mV)	RSD%	Confidence interval at (95%) $\bar{y}i \pm t0.05/2,n-1\ \sigma n-1/\sqrt{n}$	Base width Δt_B (sec)	t* (sec)	V* (ml)	Concentration in mMol.L^{-1} at flow cell
0	0	520	0.22	520±2.842	30	22	1.667	1.121
30	0.235	496	0.27	496±3.327	34	30	1.854	1.008
60	0.471	432	0.32	432±3.434	40	36	2.134	0.876
100	0.785	392	0.36	392±3.506	46	42	2.414	0.774

t* =Departure time for sample segment from injection valve to the measuring cell
V*= Final volume of segment at flow cell
C* = Concentration of sample segment(70mMol.L^{-1}) at flow cell

Variable Intensity of Light (6LEDs)

Intensity of light source was studied using 70 mMol. L^{-1} of $K_3[Fe(CN)_6]$. While 267 µl sample volume of 7mMol.L^{-1} CFTS,1.6 and 1.2 mL.min^{-1}flow rate for carrier stream and reagent respectively with open valve mode. Variable intensity of incident light source was used ranging 1.05- 2.00V by variation of light intensity knob in Linear Array Ayah 6SX1-T-1D-CFI Analyser operation where read by Avo-meter**. Fig.12A** shows the profile. The results are tabulated in **Table.11** which shows the continuation for the increase in attenuation of incident light with increasing of intensity of incident light. So 1.85 Volt DC. was selected as the optimum voltage for the snow white light emitting diode, that can be supplied to give a better reproducible outcome as shown in **Fig.12 B** .

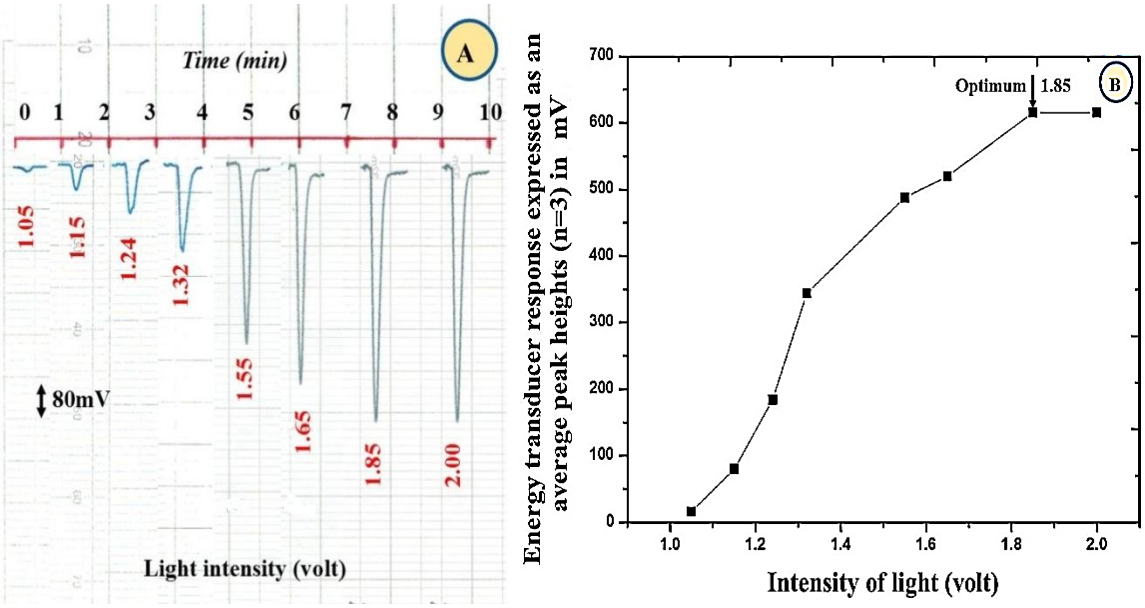


Fig. 5.12: Effect of the variation of light intensity on the:
(A): Response profile versus time,
(B): Energy transducer response expressed as an average peak heights in mV.using CFTS-$K_3[Fe(CN)_6]$ system & Ayah 6SX1-T-1D Solar cell CFIA

Table 5.11: Effect of intensity of light on the energy transducer response using CFTS-$K_3[Fe(CN)_6]$ system

Intensity of light (Volt)	Energy transducer response expressed as an average peak heights (n=3) $\bar{y}_i$ in (mV)	RSD%	Confidence interval at (95%) $\bar{y}_i \pm t_{0.05/2,n-1}\, \sigma_{n-1}/\sqrt{n}$
1.05	16	8.06	16±3.204
1.15	80	1.56	80±3.100
1.24	184	0.65	184±2.971
1.32	344	0.34	344±2.906
1.55	488	0.24	488±2.910
1.65	520	0.22	520±2.842
1.85	616	0.18	616±2.755
2.00	616	0.18	616±2.755

Scatter Plot Calibration Curve for Variation of CFTS versus Energy Transducer Response

Using the optimum chemical and physical parameters; a series of CFTS solution (1-50 mMol.L^{-1}) were prepared. Each measurement was repeated in triplicate. **Fig.13A** shows responses profile & height for each CFTS concentration .A scatter plot diagram shows that a linear calibration graph range for the variation of the energy transducer response of Ayah 6SXI-T-1D solar cell CFI Analyser with CFTS concentration was ranging from 1-50 mMol.$L^{-1}$with correlation coefficient (r): 0.9997 as shown in **Fig.13B.** While **Fig.13 C** represent the calibration graph of CFTS using conventional spectrophotometric method via measurement of λ_{max} at 260nm [9]. Low level concentration (0.03-0.35) mMol.L^{-1} with correlation coefficient (r):0.9979 can be used in the conventional method. All results were summarized in **Table.12** in which**,** tabulate the estimated value of the response obtained from the linear regression equation.

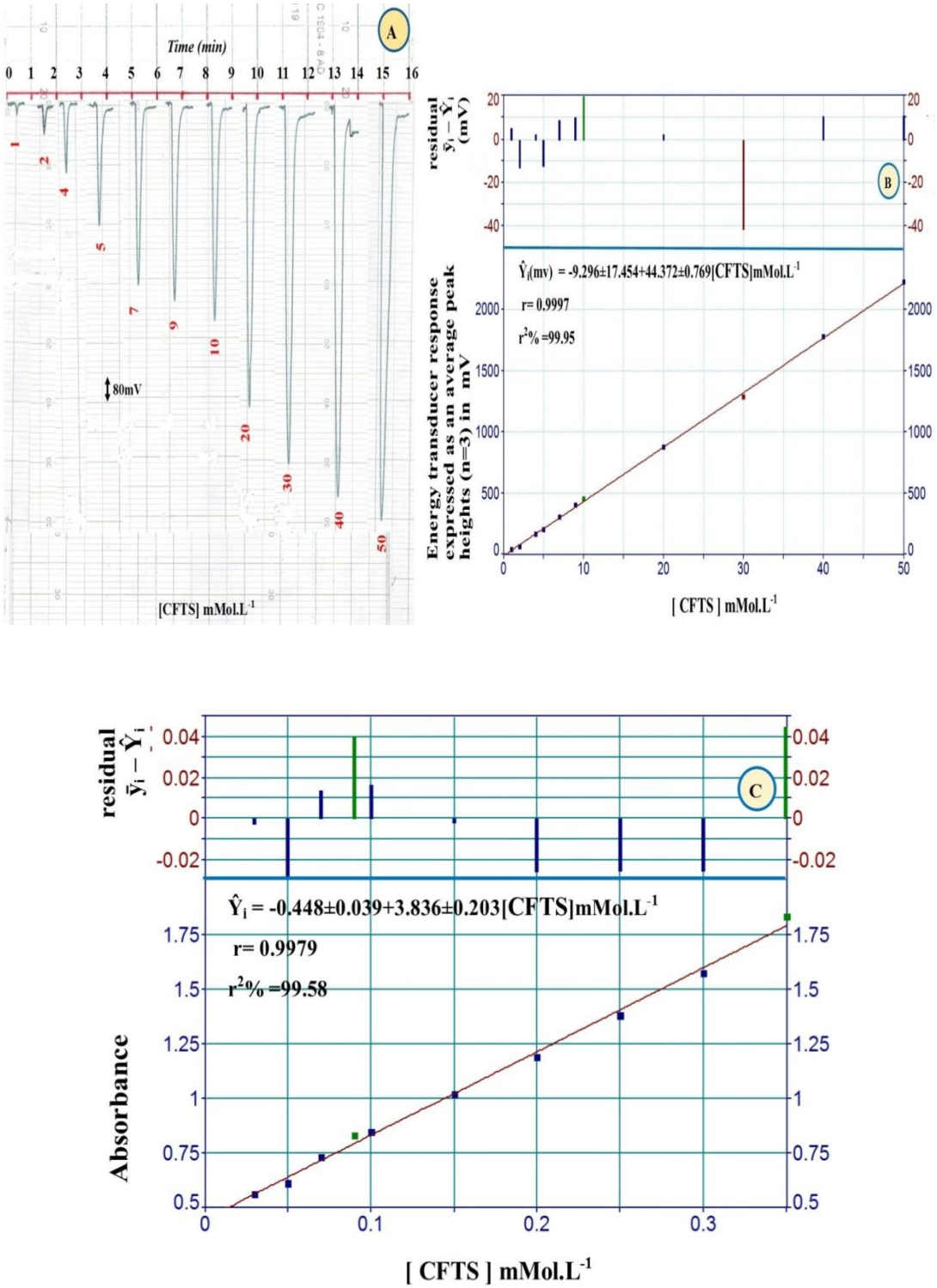


Fig. 13: (A): Selected peak profile each out of three responses for any single concentration was chosen using CFTS (7 mMol.L^{-1}) in aqueous medium , 267 µl ,1.85 volt and open valve mode

(B): Calibration graph using linear regression equation as form ŷ=a+bx of variation of response with concentration.

(C): Calibration graph using UV-Spectrophotometric method.

residual ($\bar{y}_i$ – $\hat{Y}_i$), $\bar{y}_i$: practical value, $\hat{Y}_i$: estimate value.

Table 12: Summary of result for linear regression for the variation of energy transducer response with CFTS concentration using first degree equation

Type of method	Measured [CFTS] $mMol.L^{-1}$	Range of [CFTS] $mMol.L^{-1}$ (n)	$\hat{Y}_{i(mV)}=a\pm s_a t+b\pm s_b t$[CFTS]$mMol.L^{-1}$ at confidence level 95%,n-2 $\hat{Y}_i=a\pm s_a t+b\pm s_b t$[CFTS]$mMol.L^{-1}$ at confidence level 95%,n-2	r r^2 r^2%	t_{tab} at 95% ,n-2	Calculated t-value $t_{cal}=\frac{\lvert r\rvert\sqrt{n-2}}{\sqrt{1-r2}}$
Ayah6 xs1-T-	1-50	1-50 (11)	-9.296±17.454+44.372±0.769[CFTS]$mMol.L^{-1}$	0.9997 0.9995 99.95%	2.262 << 130.634	
UV-Spectrophotom	0.03-0.35	0.03-0.35 (10)	0.448±0.039+3.836±0.203 [CFTS]$mMol.L^{-1}$	0.9979 0.9958 99.58%	2.306 << 34.618	

Ŷ=estimate value , r = correlation coefficient, r^2= coefficient of determination (C.O.D), **r^2% =** Linearity percentage. $t_{tab} = t_{0.025\ ,n-2}$

Limit of Detection (L. O. D)

The gradual dilution of minimum concentration for CFTS in the calibration graph was used for the determination the practical limit of detection of two different method. **Table.13** tabulated the limit of detection of CFTS using three methods for it is expression: gradual dilution, based on the numerical value of slope and from the linear regression using 267µL sample volume.

Table 13: Summary of limit of detection based on different approaches at 267µL sample volume

Type of method	Practically based on gradual dilution for the minimum concentration ($mMol.L^{-1}$)	Theoretical based on the value of slope $X=3S_B/Slope$	Theoretical based on the linear equation $\hat{Y}=Y_B+3S_B$
	weight/267µL		
Attenuation of incident light using Ayah6SX1-T-1D Solar cell	(0. 5 $mMol.L^{-1}$) 63.739µg/sample	3.471 µg/sample	154.785 µg/sample
Absorbance using UV-spectrophotometric	(0.02 $mMol.L^{-1}$) 38.196µg/sample	0.597 mg/sample	43.314µg/sample

SB: standard deviation of blank solution repeated for 13 times, X= value of L.O.D based on slope.
YB: average response for the blank solution (equivalent to intercept in straight line equation)

Repeatability

The repeatability of measurements and the efficiency of homemade Linear Array Ayah 6SX1-T-1D-solar cell CFI analyser were studied at fixed concentrations of CFTS (mainly two concentrations of 9 and 20 $mMol.L^{-1}$) using the optimum parameters. A repeated measurements for eight and six successive injections were measured **Fig.14A,B** shows a kind of response profile for the used concentrations. While the obtained results are tabulated in **Table.14** which shows that the percentage relative standard deviation was less than 1%.

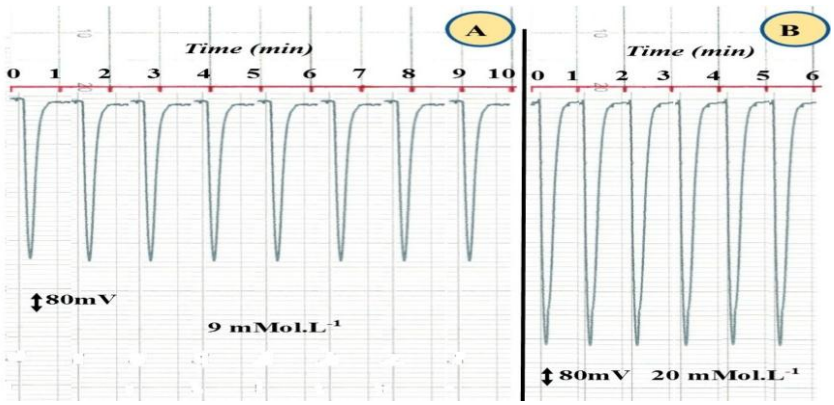


Fig. 14: Response profile -time of :
(A): Eight successive repeatable measurements of CFTS concentration (9 $mMol.L^{-1}$)
(B): Six successive repeatable measurements of CFTS concentration(20 $mMol.L^{-1}$)

Table 14: Repeatability for the response obtained for the formation of precipitation reaction from CFTS-$K_3[Fe(CN)_6]$ (7mMol.L^{-1}) system with 267 µl sample volume

[CFTS] mMol.L^{-1}	Number of injection	Average response $\bar{y}_{i\ (mV)}$	σ_{n-1}	RSD %	Confidence interval at (95%) $\bar{y}_{i(mV)} \pm t_{0.05/2,n-1}\ \sigma_{n-1}/\sqrt{n}$
9	8	664	1.20	0.18	664 ±1.003
20	6	1024	1.12	0.11	1024 ±1.176

$t_{0.025,n-1}$

$t_{0.025,5}$=2.571, $t_{0.025,7}$= 2.365

Analysis of pharmaceutical preparation

The established two methods were used for the determination of CFTS in three different samples of 1g CFTS from three different drug manufactures (Claforan-Sanofi aventis-France), (CEFOTAXIME-*L D P*-Spain), and (CETAX-AUROBINDO-India) using (Ayah 6SX1-T-1D solar cell CFI Analyser with six snow white light emitting diodes as a source for measuring turbidity via attenuation of incident light and UV-spectrophotometric at λ_{max} = 260nm .A series of solutions were prepared of each pharmaceutical drug (50 mMol.L^{-1},2.3873g (2387.24 mg) of active ingredient in 100 ml) by transferring 1mL to each of the five volumetric flask (10 mL), followed by the addition of gradual volumes of standard CFTS (0, 1, 1.2, 1.4 & 1.6) mL of 50mMol.L^{-1} to obtain (0, 5, 6, 7 & 8) mMol.L^{-1}. flask no.1 is the sample for applied standard addition method. The measurement were conducted using Ayah 6SX1-T-1D solar cell CFI Analyser method, while transferring 0.01ml to each five volumetric flask 10 ml, followed by the addition of gradual volumes of standard solution of CFTS (50mMol.L^{-1}) (0,0.01,0.014,0.018&0.02) ml to obtain (0,0.05,0.07,0.09&0.1) mMol.L^{-1} for the classical method (UV-spectrophotometric at λ_{max}= 260nm) [9]. The measurements were conducted by both methods. **Fig.15A,B,C,D** shows profile versus time and standard addition calibration graphs using developed method for three different drugs. While **Fig.16A,B,C** standard addition calibration graphs using classical method .The results were summed up in **Table.15 A**, at confidence level 95%, showing practical concentration for each pharmaceutical preparations using two method of analysis.

Table.15 B, was shown a practical content of active ingredient expressed as an average of weight in mg and efficiency of determination in addition to paired t-test at two different comparison:-

First: Individual t-test: Comparing individual between mean ($\bar{w}_i$) which represented the practically content of CFTS with quoted value (µ) [British pharmacopeia] [10]. **Table.15 B** Column 8 was shown individual dependent t-test[11-13]. Three drugs of three different companies and manufacturer were used : Claforan -France, CEFOTAXIME- Spain& CETAX-India Assuming the following assumptions :

Null hypothesis : $H_\circ$ Claforan -France $\bar{w}_i = \mu$ (1g)
CEFOTAXIME- Spain $\bar{w}_i = \mu$ (1g)
CETAX-India $\bar{w}_i = \mu$ (1g)

Against

Alternative hypothesis: H_1 Claforan -France $\bar{w}_i \neq \mu$ (1g)
CEFOTAXIME- Spain $\bar{w}_i \neq \mu$ (1g)
CETAX-India $\bar{w}_i \neq \mu$ (1g)

The obtained results indication clearly that there was a significant difference between practical content using newly developed method with quoted value at 95% confidence level as the calculated t-value is greater than t-value (4.303) at degree of freedom = n-1 , that mean; the Null hypothesis will be rejected and accepted Alternative hypothesis ; which mean that there is a significant difference between quoted value and practical content of active ingredient ;which might be due to interference effect.

Secondary

A paired t-test was conducted between the sample from three different manufacturers by either method of analysis i.e: using Ayah 6SX1-T-1D solar cell-CFI Analyser with classical method as shown in **Table.15.B** column 11 follows:

Null hypothesis :$H_\circ$: $\mu_{\text{Ayah 6SX1-T-1D solar cell-CFI}} = \mu_{\text{UV-spectrophotometric}}$

against

Alternative hypothesis :

$H_1 = \mu_{\text{Ayah 6SX1-T-1D solar cell-CFI}} \neq \mu_{\text{UV-spectrophotometric}}$

Since $t_{calculate}$ =| - 0.299| ≪(4.303),therefore , $H_{\circ}$ is accepted against H_1.These indicated, there is no significant different between two methods.That conclude any of the methods can be used equally and satisfactorily for the analysis of any of the drugs **.**

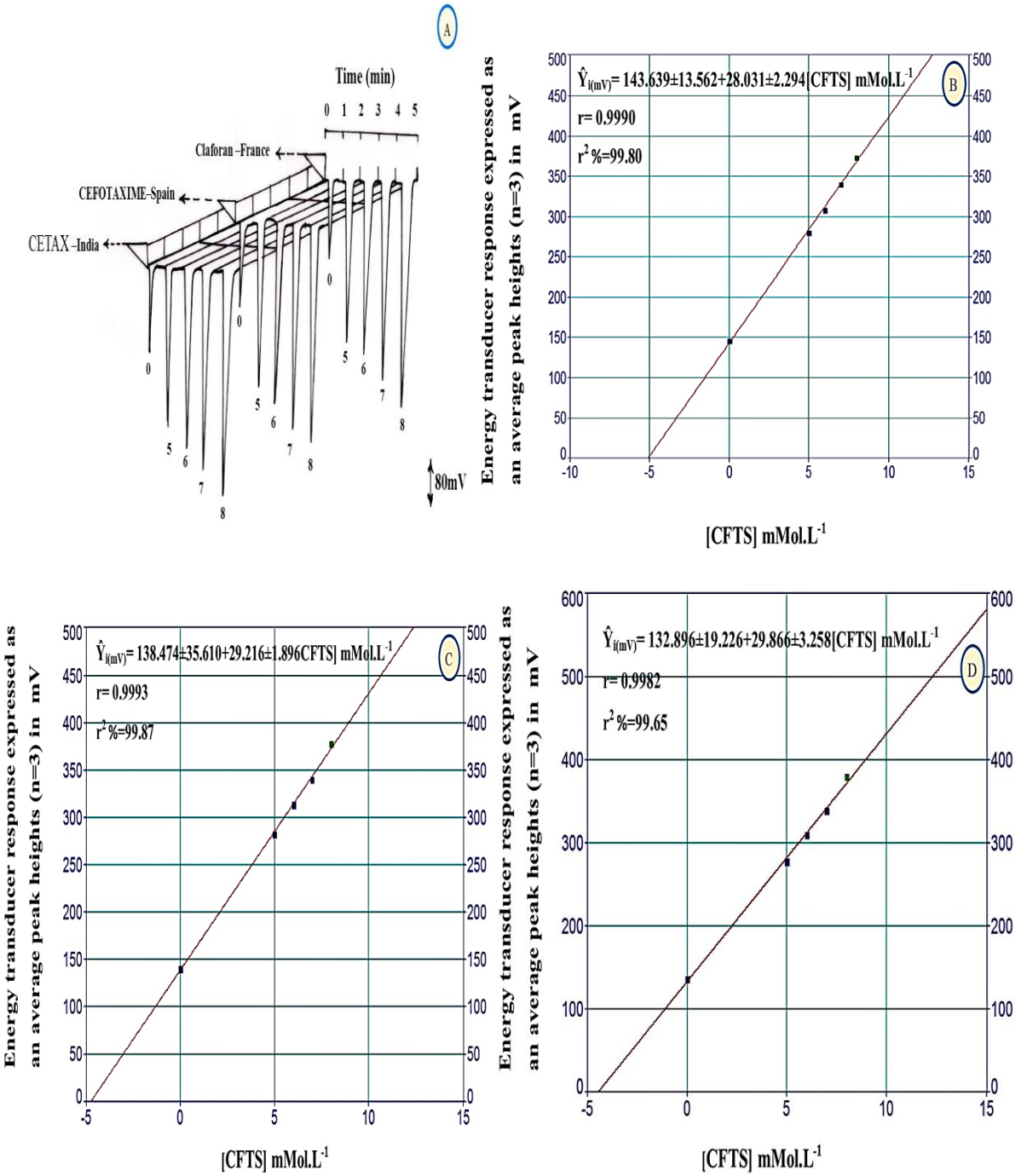


Fig. 15: Standard addition calibration graph and sample of response profile versus time for three pharmaceutical preparations
(A): Profile versus time
(B): Standard addition calibration graphs, Claforan-France
(C): Standard addition calibration graphs, CEFOTAXIME- Spain
(D): Standard addition calibration graphs, CETAX-India using developed method

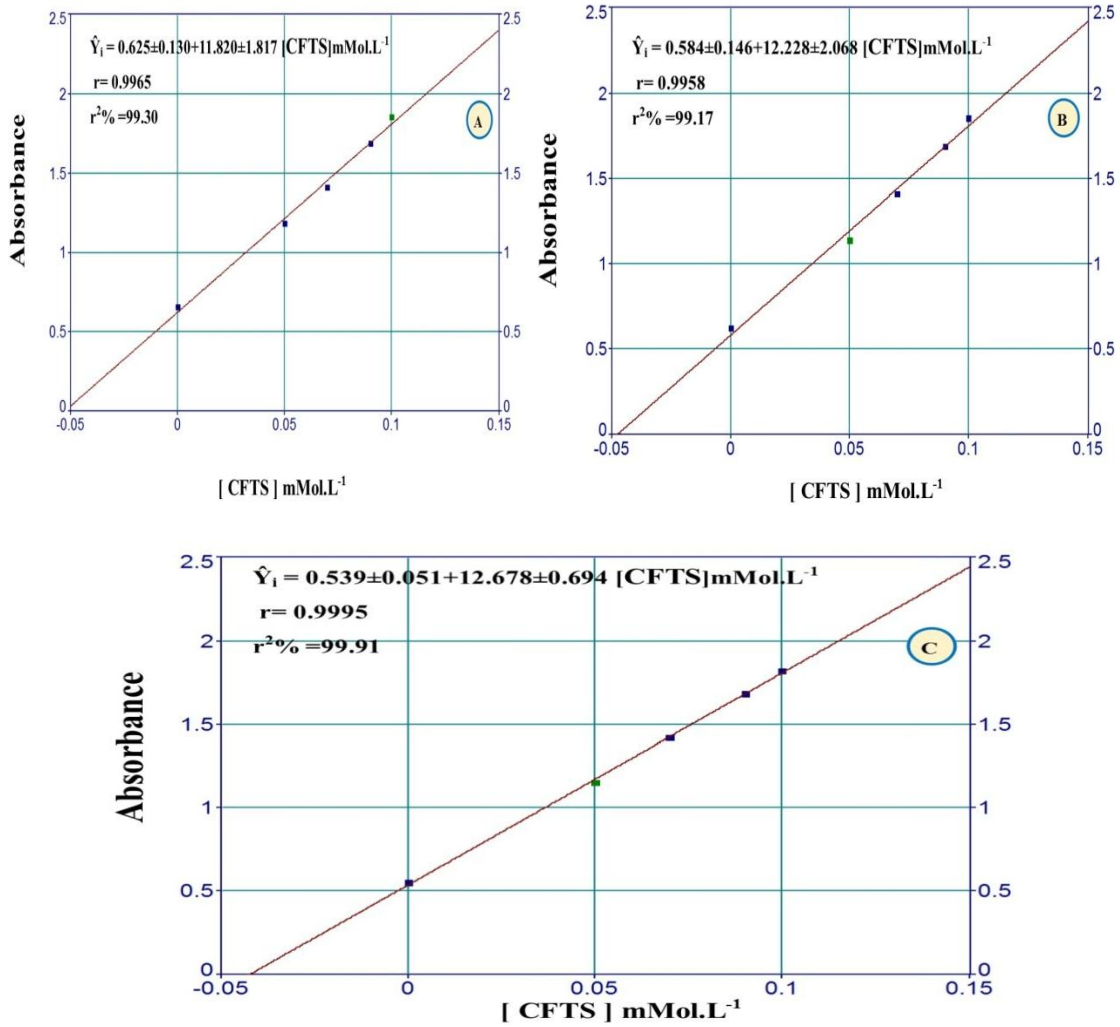


Fig. 16: Standard addition calibration graph using classical Method (UV- spectrophotometric), using three different drugs. (A): Claforan-France, (B): CEFOTAXIME- Spain and (C): CETAX-India

Table15 A: Results for the determination of CFTS in pharmaceutical preparations by standard addition method using Ayah 6SX1- T-1D Solar cell CFI Analyser & Classical method (UV- Spectrophotometric)

Sample no	commercial name , content company, country	*Ayah 6SX1-T-1D solar CFIA* *UV- Spectrophotometric (classical method for absorbance measurement)* [CFTS] mMol.L^{-1}					Equation of standarad addition curve at 95% for n-2 $\hat{Y}_{i(mV)}=a\pm s_a t+b\pm s_b t$[CFTS] mMol.L^{-1} $\hat{Y}_i^*=a\pm s_a t+b\pm s_b t$[CFTS] mMol.L^{-1}	r r^2 r^2%	Practical conc. mMol.L^{-1}
		0	5	6	7	8			in 10 ml
		0	0.05	0.07	0.09	0.1			In100ml
1	Claforan 1g Sanofi aventis FRANCE	146	280	308	340	373	143.639±13.562+28.031±2.294[CFTS] mMol.L^{-1}	0.9990 0.9980 99.80%	5.124 51.24
		0.655	1.181	1.411	1.687	1.855	0.625±0.130 + 11.820± 1.817 [CFTS] mMol.L^{-1}	0.9965 0.9930 99.30%	0.053 53
2	CEFOTAXIME 1g L D P SPAIN	140	282	313	340	377	138.474±35.610 + 29.216±1.896[CFTS] mMol.L^{-1}	0.9993 0.9987 99.87%	4.740 47.40
		0.622	1.138	1.411	1.687	1.855	0.584 ±0.146 + 12.228±2.062 [CFTS] mMol.L^{-1}	0.9958 0.9917 99.17%	0.048 48

3	CETAX 1g AURBINDO INDIA	136	277	309	339	380	132.896±19.226+29.866±3.258[CFTS] mMol.L^{-1}	0.9982 0.9965 99.65%	4.450 44.50
		0.552	1.151	1.420	1.682	1.821	0.539 ± 0.051+12.678 ± 0.694 [CFTS] mMol.L^{-1}	0.9995 0.9991 99.91%	0.043 43

$\hat{Y}_i^*$= estimated value for absorbance, r= Correlation coefficient , r^2= coefficient of determination (C.O.D), r^2%= Linearity percentage

Table.15 B: Summary of data for paired t-test, practical content and efficiency of determination of CFTS in three samples pharmaceutical preparation

Sample no	Confidence interval for the average weight of tablets $\bar{w}_i \pm 1.96\ \sigma_{n-1}/\sqrt{n}$ at 95% (g)	Theoretical content for the active ingredient $\bar{w}_i \pm 1.96\ \sigma_{n-1}/\sqrt{n}$ at 95% (mg)	Sample weight equivalent to 2.387 g (50 mMol.L^{-1})of the active ingredient w_i (g)	Practical content of active ingredient		Efficiency of determination (Rec%)	Paired t-test			
				In100 ml of sample $\bar{w}_i \pm t_{0.05/2,n-1}\ \sigma_{n-1}/\sqrt{n}$ at 95%, (mg)	In vials $\bar{w}_i \pm t_{0.05/2,n-1}\ \sigma_{n-1}/\sqrt{n}$ at 95% ,(mg)		Individual comparison $(\bar{w}_i - \mu_o)\sqrt{n}/\sigma_{n-1}$ Ayah 6SX1-T-1D Solar cell-CFI Analyser with Quoted value $t_{0.05/2,2}$=4.303	Comparison between two method		
				Ayah 6SX1-T-1D solar CFIA (mV)						
				UV- SP (classical method for absorbance measurement)				Xd	$\overline{Xd}$ (σ_{n-1})	$t_{cal} = \overline{Xd}\sqrt{n}/\sigma_{n-1}$ at 95 %
1	1.046±0.002	1000 ±1.912	2.497	2446.438±4.223	1025±1.769	102.5%	60.811 ≫ 4.303	-35	-5.692 32.992	-0.299 ≪ 4.303
				2530.469±4.348	1060±1.821	106%				
2	0.986± 0.036	1000 ±36.511	2.354	2263.099±5.167	948±2.164	94.8%	|-103.399| ≫ 4.303	-12.116		
				2291.746±5.068	960.116±2.123	96.012%				
3	1.023±0.008	1000±7. 820	2.443	2124.639±4.894	890±2.050	89%	|-230.893|≫4.303	30.04		
				2053.022±4.969	859.960±2.081	85.996%				

Xd **:** Difference between two method , $\overline{Xd}$: difference mean , σ_{n-1}**:**Difference standard deviation , n=3 for individual & n=2 for comparison between two method, μ (quoted value =1g) & $\bar{w}_i$: average weight

In addition to use another hypothesis about the effect of samples supplied by three different companies on the results of analysis using one-way ANOVA[14,15] which was carried out ∝ = 0.05 (95% confidence level). The results obtained for determination of CFTS by three methods (standard, UV-spectrophotometry and turbidimetry using Ayah 6SX1- T-1D Solar cell CFI Analyser)from three different companies(Claforan -France, CEFOTAXIME- Spain& CETAX-India) summarize in **Table 16.**

Table 16: Summed up results for three different methods and different companies for analysis of variance of CFTS

No. of sample	Claforan - FRANCE (1)	Cefotaxime-SPAIN (2)	CETAX- INDIA (3)
Standard method Quoted value	1000	1000	1000
Classical method UV-spectrophotometer At λmax=260nm	1060	960.116	859.960
Developed method Ayah 6SX1-T-1D -Solar cell Analyser	1025	948	890

The following hypothesis should be used:

H_o (Null hypothesis)

$$\mu_{\text{Claforan –France}} = \mu_{\text{CEFOTAXIME- Spain}} = \mu_{\text{CETAX-India}}$$

Against

H_1 (Alternative hypothesis)

$$\mu_{\text{Claforan –France}} \neq \mu_{\text{CEFOTAXIME- Spain}} \neq \mu_{\text{CETAX-India}}$$

Table 17 showing the effect between three different supplier companies on the measurements using one way ANOVA by calculation sum of squares (Ssq) degree of freedoms (D_f), mean squares (Msq), F-value and the significant test results.

Table 17: Analysis of variance for three different samples of CFTS

Source	Sum of squares (Ssq)	D_f	Mean square (Msq)	F_{value}	F_{tab}	Sig
Between groups	18615.78	2	9307.89	3.942<< 5.14		0.105>>0.05 No significant
Within groups	14168.38	6	2361.39			
Total	32784.16	8				

$$F_{tab} = F_6^2 = 5.14$$

The analysis results shows, the value of sig (0.105) >> 0.05 and $F_{cal} << F_{tab}$, therefore Null hypothesis will be accepted and will rejected the Alternative hypothesis .These mean that there is no significant difference between the means of the different companies supply different drugs samples.

CONCLUSION

The suggested methods is simple, sensitivities and rapid. Application of the proposed methods to the analysis of Cefotaxime sodium in pharmaceutical preparation based on formation yellowish white color precipitate as an ion- pair compound for the reaction of CFTS - K_3 [Fe $(CN)_6$] in aqueous medium. It was shown that with no doubt that newly developed method is a good as the classical method. An alternative analytical method is found through this research work, which based on simple parameter conditions.

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