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RESEARCH ARTICLE

Semiautomated On-line for the Determination of Sitagliptin Phosphate using UV-low Pressure Mercury Lamp at 184.9 nm and 253.7 nm at ISNAG-fluorimeter Analyzer

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Abstract

New methodology for sitagliptin phosphate determination via on-line continuous flow injection manifold design of sitagliptin phosphate-phosphomolybdic acid (PMA) system. The method was applied using continuous flow injection system of a new homemade ISANG -fluorimeter with turbidity measurements at 90^0 via 2×4 solar cell. Chemical and physical parameters were studied and optimized. The calibration graph was the linear dynamic in the range of (0.1-4.5 mmol.L^{-1}) which was better representation, with correlation coefficient r=0.9965. The limit of detection (LOD) 35.999µg/sample from the stepwise dilution for the minimum concentration in the linear dynamic ranged of the calibration graph. The method was successfully applied for the determination of sitagliptin phosphate (STP) in two pharmaceuticals preparation of different companies. A comparison was made between the newly developed method analysis with the classical method (UV- spectrophotometry at λ max =267 nm) using the standard addition method via the use of individual and paired t-test and F-test. It was noticed that there was a significant difference between two methods at 95 % confidence level.

Keywords: *Sitagliptin phosphate, Turbidity, Flow injection analysis.*

Introduction

Sitagliptin phosphate (STG) is an oral drug hypoglycemic agent (anti-diabetic) drug [1]. Sitagliptin phosphate it has described chemically 1,2,4-triazolo[4,3-a]pyrazine,7-[(3R)- 3- amino- 1- oxo- 4- (2,4,5-trifluorophenyl)butyl]- 5, 6, 7, 8- tetrahydro-3(trifluoromethyl), phosphate [2,3]. The structure is given in Fig.1.

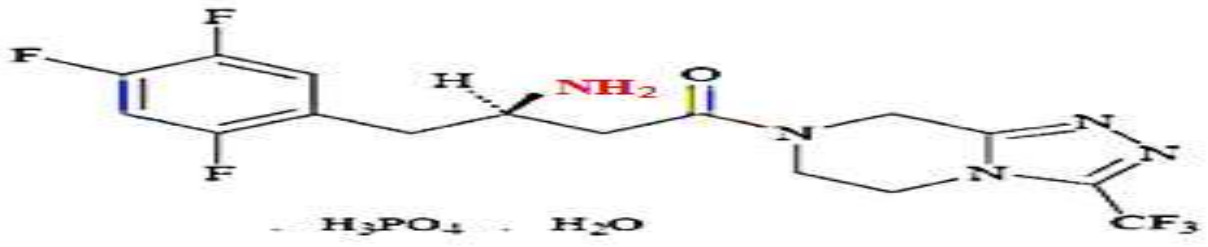


Figure 1: Chemical structure of sitagliptin phosphate monohydrate

Sitagliptin Phosphate is act as blocks selectively the dipeptidyl peptidase 4 (DPP-4) [4] inhibitor class, this enzyme reduces the breakdown of GLP-1 and increases insulin secretion [5], this suppresses the liberation of glucagon from the pancreas and led to drives down blood sugar levels.

The STG drug enzyme-inhibiting is used either alone or in combination with other oral antihyperglycemic agents (such as metformin or thiazolidinedione) for treatment of diabetes mellitus type 2. The interest using of this drug is its lower side-effects (e.g., less hypoglycemia, less weight gain) in the control of blood glucose values [6].

Sitagliptin phosphate monohydrate (STP) is a (white-off-white, crystalline, non-hygroscopic powder. It is soluble in water and N, N-dimethyl formamide; very slightly soluble in ethanol, acetone, and acetonitrile; slightly soluble in methanol; and insoluble in isopropanol and isopropyl acetate. Many methods have been reported for the determination and study of STP in pure form and pharmaceutical preparation, depended on developed instrumental techniques, which includes: spectroscopy [7], Spectroflourimetric [8], RP-HPLC [9], HPTLC [10], UPLC [11], capillary zone electrophoresis [12] and Mass spectroscopy [13].

The aim of this study was to develop a new fluorimetric continuous flow injection method for the determination of sitagliptin phosphate (STP) using the homemade ISANG-fluorimeter [14] .This method based on precipitation sitagliptin phosphate (STP) via PMA (as precipitating agent) , this formed precipitate will be irradiated by two main wavelength using low pressure mercury lamp at 184.9nm and 253.7 nm (prominent).All kind of scattered light will be detected at 90^0 through a path of 2mm optical operture extended for 100 mm distance via the use of two side solar cell, each side contain four solar cells (i.e; 2[4x2.5cm (length)].

Materials and Methods

Apparatus and Reagents

Apparatus

A homemade ISANG fluorometer was used with multichannel more than one line feed (In this part of the research work only two lines) were used, four-channels peristaltic pump (Ismatec, Switzerland) and Six-port medium pressure injection valve .The output of measurement i.e; $\bar{Y}z_i$(mV)-t_{min}(d_{mm})was plotted by potentiometric recorder was used to estimate the output signals (Siemens, Germany (1- 5 V)). Spectrophotometer (UV-1800, shimadzu, Japan) was used as classical methods.

Reagents and Solutions

All employed chemicals were used of analytical -reagent, distilled water was used to prepare all the solutions. A standard solution of 12 mmol.L^{-1} and 50 mmol.L^{-1} of PMA and STP, molecular weight 1825.25 and 523.32 g.$mole^{-1}$ respectively, were prepared by dissolving 5.4757 g of PMA in 250 mL of distilled water and also dissolving 6.5414 g of PMA in 250 mL of distilled water. Aqueous solutions of different medium of salts (100mmol.L^{-1}) NaCl, KBr, NH_4Cl, CH_3COONH_4 and $NaNO_2$ solutions were prepared by dissolving 0.585g of NaCl (M.Wt=58.5 g.mol^{-1}) in 100 ml distilled water,1.19002g of KBr(M.Wt=119.002g.mol^{-1}) in 100 ml distilled water, 0.535g of NH_4Cl (M.Wt=53.5 g.mol^{-1}) in 100 ml distilled water, 0.7768 g of CH_3COONH_4(M.Wt=77.683 g.mol^{-1}) in 100 ml distilled water and 0.690g of $NaNO_2$(M.Wt=69.0 g.mol^{-1}) in 100 ml distilled water .

Sample Preparation

Twenty tablets of two different pharmaceuticals drug companies (Sitavia; Pioneer and Januvia; MSD) containing 100 mg of sitagliptin phosphate were weighed, crushed and grinded .A solution of 10 mmol.L was prepared from different pharmaceuticals drugs by weighing 2.9384 and 2.1623 g quivalent to 0.523 g of active ingredient from Sitavia; Pioneer and Januvia; MSD) respectively. Each one from the two kinds of sample was dissolved in distilled water. The solution was filtered to get rid of undissolved materials; the residue was washed and completes the volume to 100 ml with distilled water.

Methodology

Sitagliptin assessment was based on the use of phosphomolybdic acid as a precipitating agent. Yellowish white precipitate is formed probably as an ion pair using two line manifold unit design (Fig.2). First line is the carrier line stream of NaCl at 70 mmol.L^{-1} concentration (2.1ml.min^{-1}) that will pass through injection valve to carry the sample segment (160 µL & open valve mode) of 5 mmol.L^{-1} STP concentration and combine with the second line (2 ml.min^{-1}) at Y-junction point that carry the reagent of phosphomolybdic acid at 2 mmol.L^{-1} concentration to form the precipitate.

This formed precipitate will be irradiated by two main wavelengths at 184.9nm and 253.7 nm (prominent). Both two lines are easily diverged due to its high frequency.

All kind of scattered light will be detected at 90^0 through a path of 2 mm optical operture extended for 100 mm distance via the use of

two side solar cell, each side contain four solar cells (i.e; 2[4x2.5cm (length)].

The proposed probable reaction is expressed in scheme.1 [15].

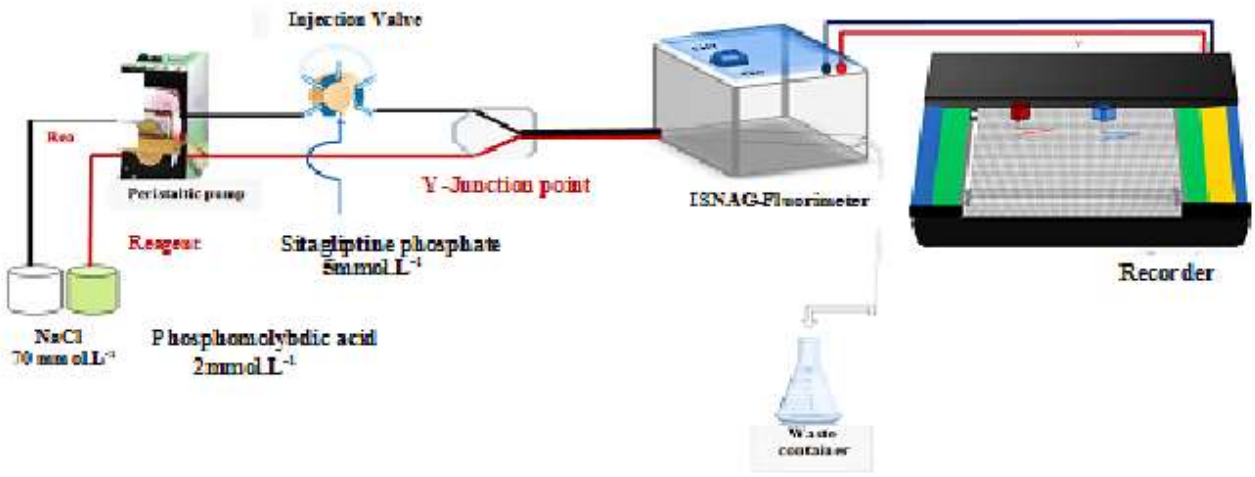
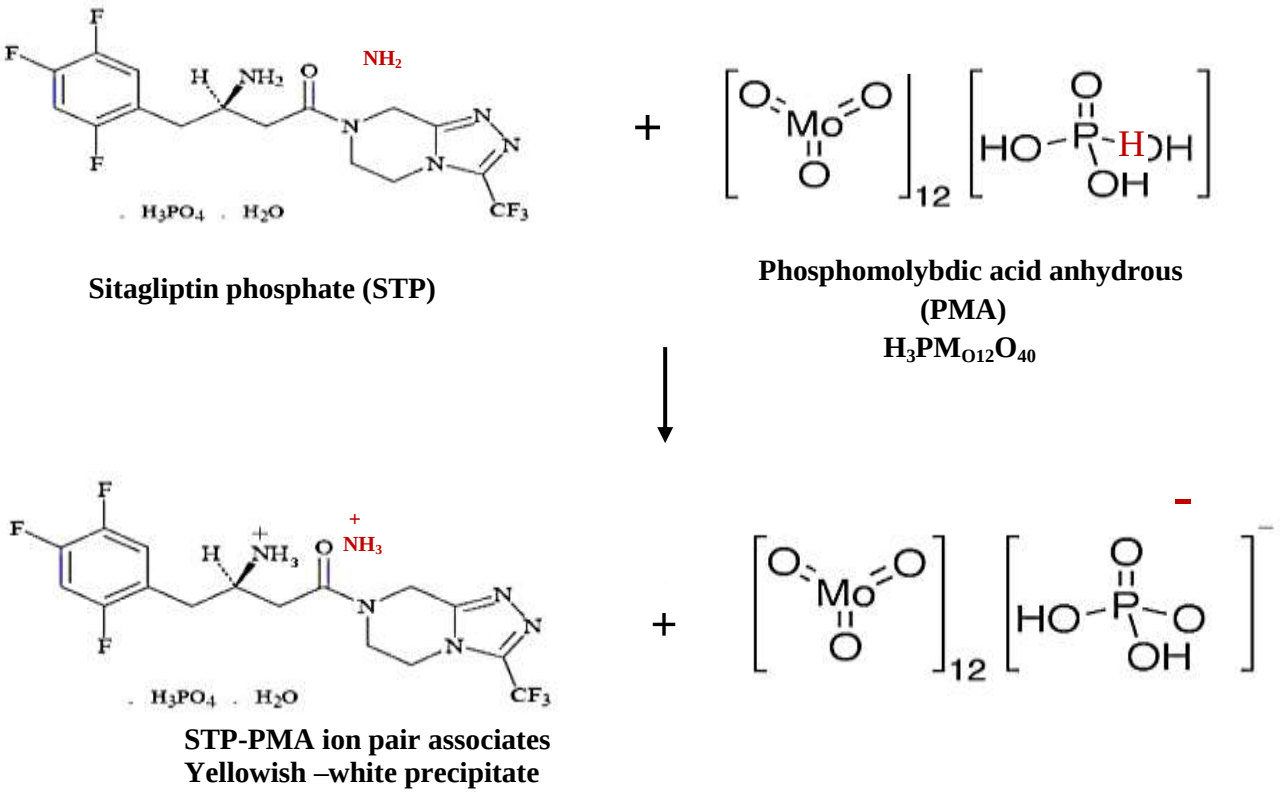


Fig. 2: Flow diagram of used manifold throughout this research work



Scheme 1: Proposed reaction between sitagliptin phosphate (STP) with phosphomolybdic acid (PMA)

Results and Discussion

Study of the Optimization of Reaction Pattern Parameters Chemical variables

Variation of Phosphomolybdic Acid Concentration on Precipitation of STP

The study was carried out using a series of solutions ranging from 0.5 to 5 mmol.L^{-1} as a precipitate reagent at 2 ml.min^{-1} flow rate; the water is a carrier stream at 2.1 ml.min^{-1} flow rate to carry the sample segment (200 µL). Fig .3. A shows the response profile. On the basis of slope-intercept calculation which

is shown in Table 1 and Fig. 3.B, the choices of segments and concentration ranges. Three chosen segment were used. It was noticed to get a wide range of concentration. Therefore on this outcome, segment 2-5 mmol.L^{-1} was the optimum due to high intercept value. So, any concentration within the chosen segment can be selected as the optimum used parameters concerning the concentration of the precipitating agent. A concentration of 2 mmol. L^{-1}which is within the chosen segment to be the most beneficial concentration.

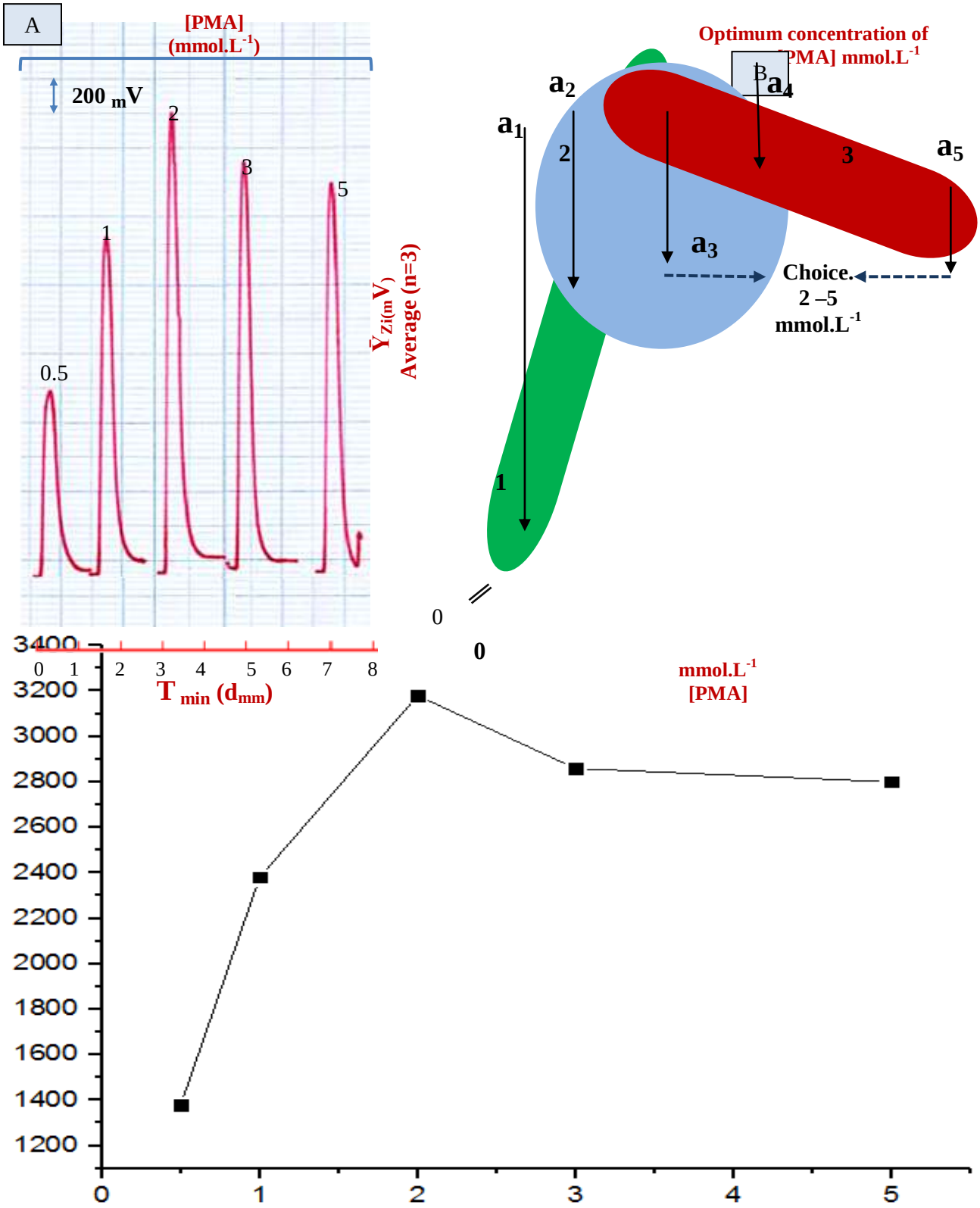


Fig. 3: A: Variation of PMA concentration on: A: Profile of PMA –STP system. B: Height of $\bar{Y}_{Zi}$(mV): (S/N) energy transducer response in mV with chosen segments of average (n=3)

Table 1: Data point and Segmentation pattern

No. of segment	[PMA]range mmol .L^{-1}	Segment	Slope mV/mmol.L^{-1}	intercept mv
1	0.5-2	a_1-a_3	1142.85	980.0
2	1-3	a_2-a_4	240.00	2367
3	2-5	a_3-a_5	-112.85	3322

Affect of Salt Solution as a Carrier Stream

The affect of different medium of salts (50 mmol.L^{-1}) NaCl, KBr, NH_4Cl, CH_3COONH_4 and $NaNO_2$ solution in addition to distilled water were studied and used as a carrier stream .This study was carried out using the optimum concentration of PMA (2 mmol.L^{-1}), 200µL sample volume and 2.1,2 ml.min^{-1} flow rate for carrier stream and PMA line respectively. It was noticed that all salts used increase the S/N energy transducer response; this might be attributed to coagulation effect. Therefore NaCl was the optimum choice to use for this system: STP-PMA as a carrier stream. The above study is represented in Fig 4.Table 2 summaries all the obtained data.

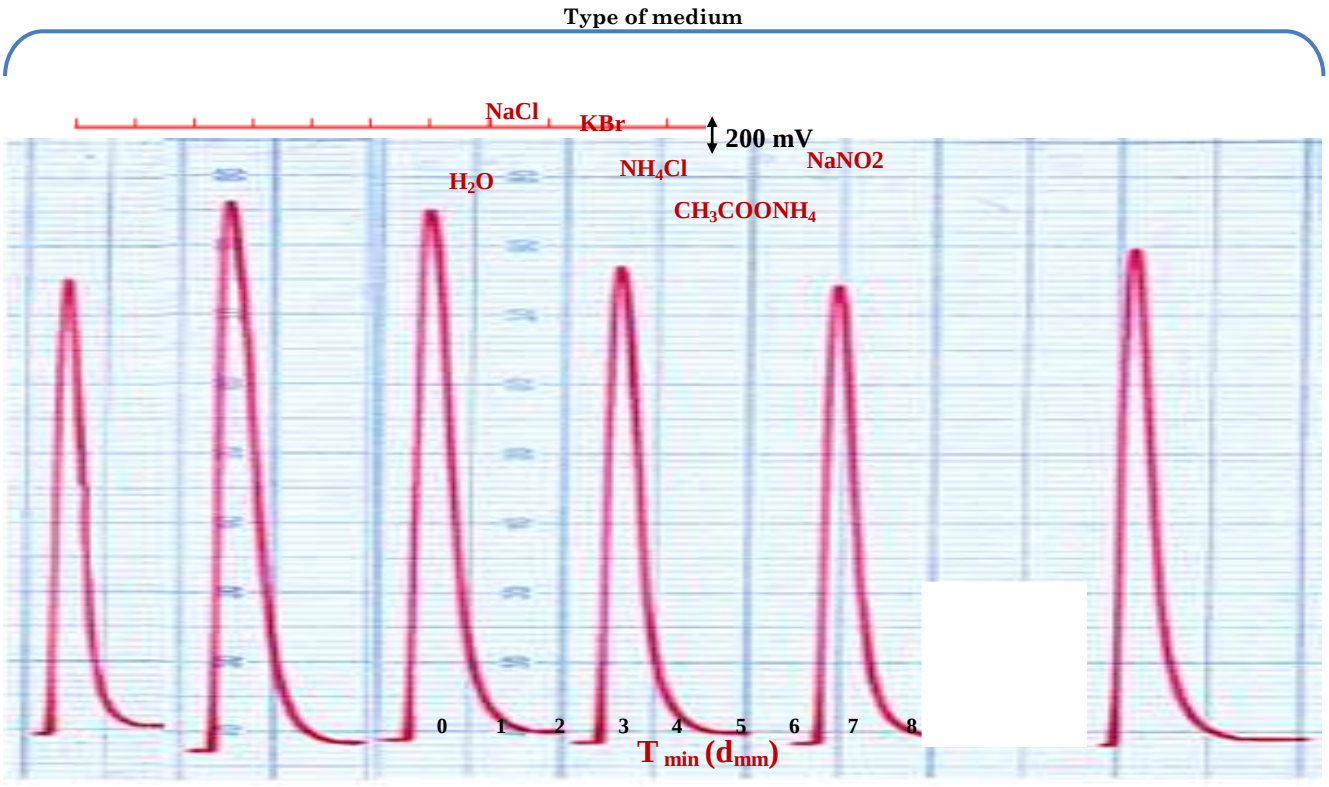


Fig. 4: Effect of different of salt concentration on: Response profile $\bar{Y}Z_i$(mV)-t_{min} (d_{mm})

Table.2: Data set point of response obtained using Different Salt media

Type of medium	$\bar{Y}_{Zi}$(mV) average (n=3)	RSD %	Confidence interval at 95% $\bar{Y}_{Zi}$(mV)±$t_{0.05/2,n-1}\sigma_{n-1}/\sqrt{n}$
H_2O	3182	0.038	3182 ± 3.006
NaCl	3820	0.035	3820 ± 3.353
KBr	3700	0.032	3700 ± 3.006
NH_4Cl	3320	0.046	3320 ± 3.776
CH_3COONH_4	3200	0.044	3200 ± 3.527
$NaNO_2$	3480	0.045	3480 ± 3.925

$t_{0.05/2,2}$=4.303, $\bar{Y}_{Zi}$ (mV) :(S/N) energy transducer response

Effect of NaCl Concentration

Sets of series solutions of sodium chloride (5-100 mmol.L^{-1}) as a carrier stream were used. It was found that an increase of peak height with increase of NaCl concentration up to 70 mmol.L^{-1}. It might be probably due to aggregation precipitating particulate and increase size of particulate this might be lead to increase of diverged light toward in the solar cells. While up to 70 mmol.L^{-1} concentration of NaCl (Table 3.A) lead to decrease of S/N energy transducer response,

most probably due to dispersion of precipitated particulate. Therefore and on the basis of slope-intercept which is shown in Table 3.B and Fig.5 in which that segment a_4-a_6 i.e.; 50-100 mmol.L^{-1} was the optimum and 70 mmol.L^{-1} within the chosen segment was the optimum carrier stream that used for further experiments.

Table 3: A- Data set point obtained for the variation of NaCl concentration in the determination procedure of STP

[NaCl] mmol.L^{-1}	$\bar{Y}Z_i(_mV)$ average (n=3)	Confidence interval at 95% $\bar{Y}Z_i(_mV) \pm t_{0.05/2,n-1}\ \sigma_{n-1}/\sqrt{n}$
5	3380	3380 ± 3.006
10	3360	3360 ± 3.353
20	3100	3100 ± 2.782
50	3820	3820 ± 3.527
70	4020	4020 ± 3.304
100	3740	3740 ± 3.105

Table 3.B: Segmentation pattern for select optimum segment of STP-PMA-NaCl system

No. of segment	[NaCl] range mmol/L^{-1}	Segment	Slope mV/mmol.L^{-1}	intercept
1	**5-20**	**a_1-a_3**	**-19.71**	**3510**
2	**10-50**	**a_2-a_4**	**14.38**	**3043**
3	**50-70**	**a_4-a_5**	**10.0**	**3320**
4	**50-100**	**a_4-a_6**	**-2.21**	**4022**

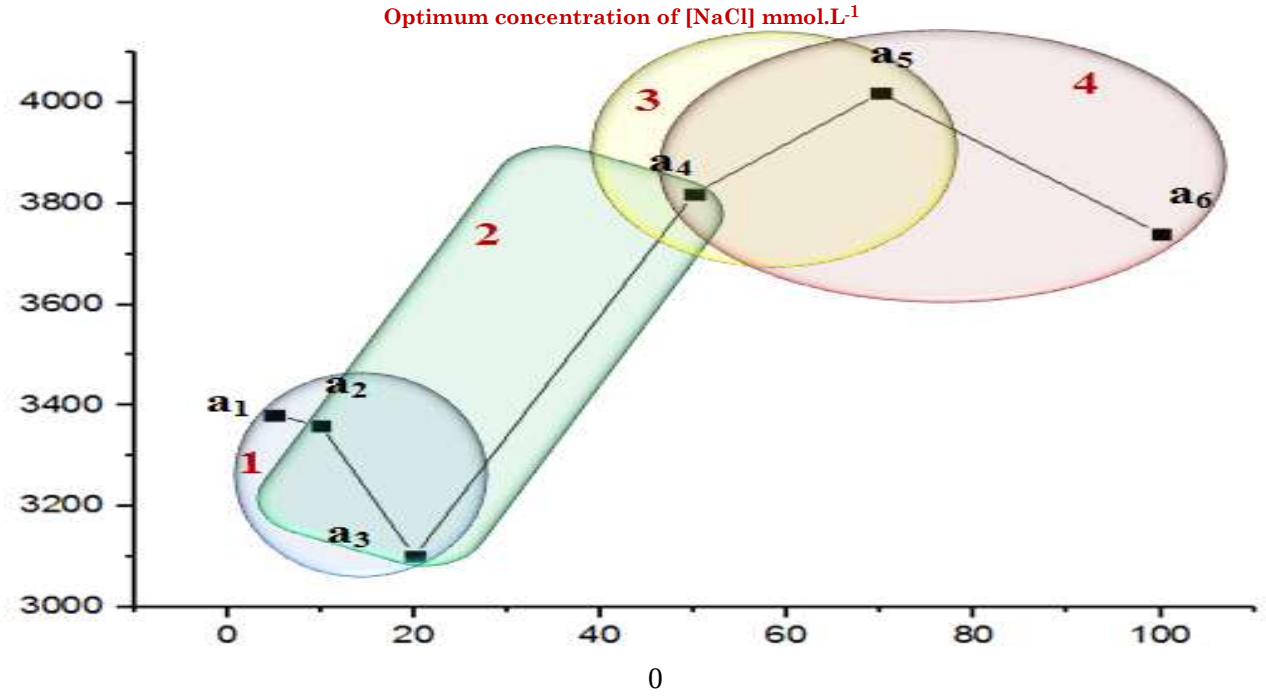


Fig. 5: Effect of variable concentration of NaCl solution on: Output of (S/N) energy transducer response in mv with chosen segments

Physical Variables

Effect of Flow rate

Variation of flow rates (0.5-3.2) ml.min^{-1} for a first line (70 mmol.L^{-1} of NaCl solution) and 0.4-3.1ml.min^{-1} for a second line (PMA line) were used for STP (5mmol.L^{-1} ,200µL)-PMA-NaCl system and open valve mode .It was noticed that at low speed; wide profile were obtained.

Most probably due to agglomeration and condensation of their masses and difficulty in moving with the carrier stream flow leading to increase of Δt_B (base width of response) Table 4.A .While at a higher speed (> 10 speed of pump), although the effect of flow rate was very crucial on the S/N energy transducer response for obtaining regular response and very sharp maxima. So, a

compromised to obtain sharp maxima of profile, minimize of consumption of reactions solution, complete precipitation and on the basis of slope - increase value of intercept calculation which is shows in the table 4.B and fig 6 (six chosen segments were used), a flow rates 2.1 and 2.0 ml.min^{-1} for line no.1 and line no.2 respectively which is within the chosen segment no.5 (i.e.; 2.1 - 2.9) were chosen as the optimum flow rate.

Table 4.A: Effect of the variation of flow rate on the measument of $\bar{Y}Z_i$(mV)(i.e.S/N energy transducer response

Pump speed	Flow rate ml.min^{-1} Line no. 1	Flow rate ml.min^{-1} Line no.2	$\bar{Y}Z_i$(mV) average (n=3)	RSD %	t* Sec	Base wid Δt_B Sec	Confidence interval at 95% $\bar{Y}z_i$(mV)± $t_{0.05/2,n-1}\sigma_{n-1}/\sqrt{n}$	V_{add} ml At flow cell	Concentration mmol.L^{-1} At flow cell
5	0.5	0.4	4700	0.026	36.0	258	4700 ± 3.105	4.07	0.246
10	1.0	0.9	4100	0.035	20.0	120	4100 ± 3.602	4.00	0.250
15	1.3	1.2	4040	0.039	18.5	114	4040 ± 3.925	4.95	0.202
20	1.7	1.6	3900	0.031	18.0	102	3900 ± 3.030	5.81	0.172
25	2.1	2.0	4000	0.033	17.5	90	4000 ± 3.279	6.35	0.157
30	2.5	2.4	3500	0.032	10.5	66	3500 ± 2.782	5.59	0.180
35	2.9	2.7	3360	0.039	9.0	54	3360 ± 3.279	5.24	0.191
40	3.2	3.1	3120	0.049	6.0	36	3120 ± 3.527	3.98	0.253

ȲZi (mV):(S/N) energy transducer response ,t*:departure time lapse from injection valve reaching to measuring cell (sec), ΔtB : Base width of peak (sec), t0.05/2,2 =4.303, line no.1: carrier stream of 70 mmol.L-1 NaCl and line no.2:PMA (2mmol.L-1)

Table.4.B: Segmentation pattern slope-intercept to select the optimum segment of flow rate (i.e.; a_5-a_7)

No. of segment	segment	Flow rate mmol.L^{-1}	Intercept mV	Slope mV/mmol.L^{-1}
1	a_1-a_3	0.5-1.3	5464	-863.26
2	a_2-a_4	1.0-1.7	1492	-289.19
3	a_3-a_5	1.3-2.1	4065	-50.00
4	a_4- a_6	1.7-2.5	4850	-500.00
5	a_5- a_7	2.1-2.9	5620	-800.00
6	a_6- a_8	2.5-3.2	4853	-532.43

Three data point as one segment and choose

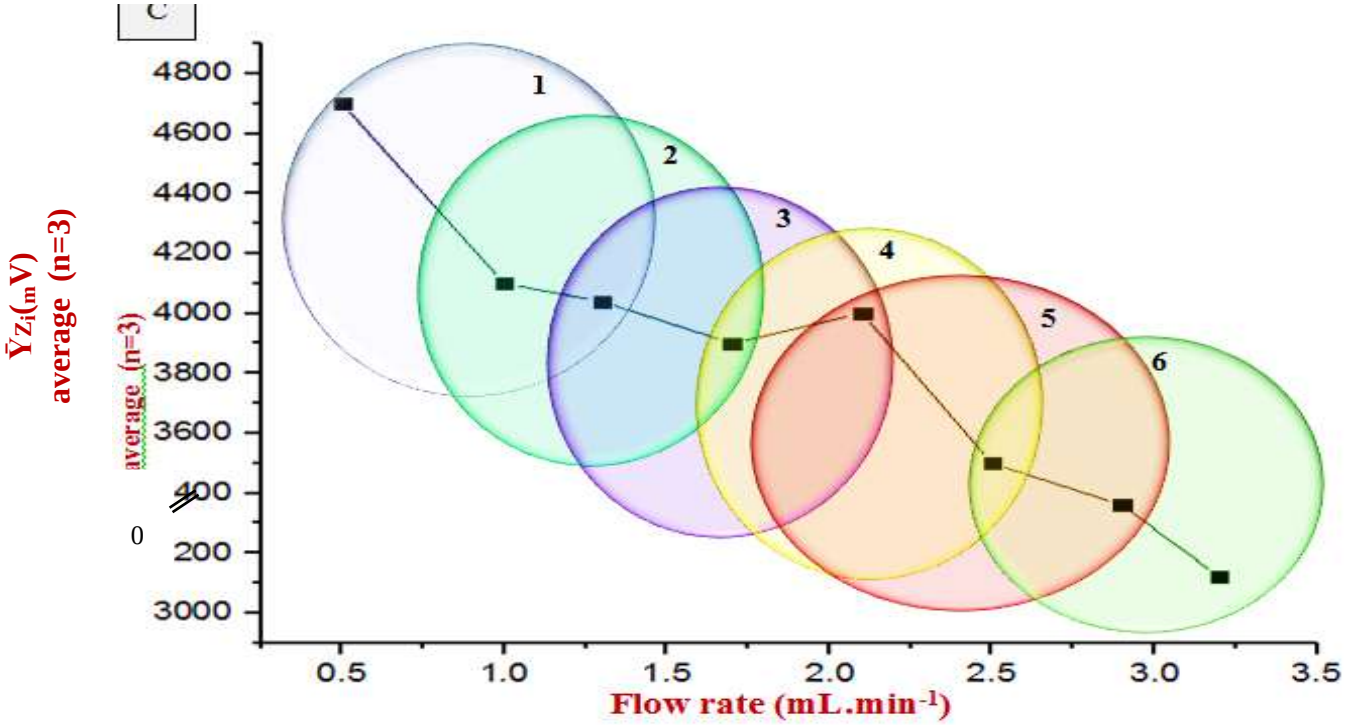


Fig.6: $\bar{Y}Z_i$(mV): Output of (S/N)energy transducer response with six of chosen segments

Variation of Sample Segment

Using STP (5mmol.L^{-1}) - PMA (2mmol.L^{-1})-NaCl (70 mmol.L^{-1}) system; the sample volume was studied. Variable sample segment ranging from 24 to 200µL were used. S/N energy transducer response is shown in Fig .7.A. It was explained that an increase in sample segment led to an increase of the peak height up to 160 µL due to larger amount of precipitate particulate formed at this volume of sample that will enhance the intensity of diverged light toward in the eight of solar cells at 0-90^{0}angle.Table 5 summarized set of data of sample volume variation. 160µL is the most satisfactory sample plug based on the above studied and on the basis of slope-intercept calculation (Table 5 and Fig.7.B) in which shows the; optimum chosen segment (96-200µL).Any sample segment within the chosen segment can be selected as the optimum used parameters.

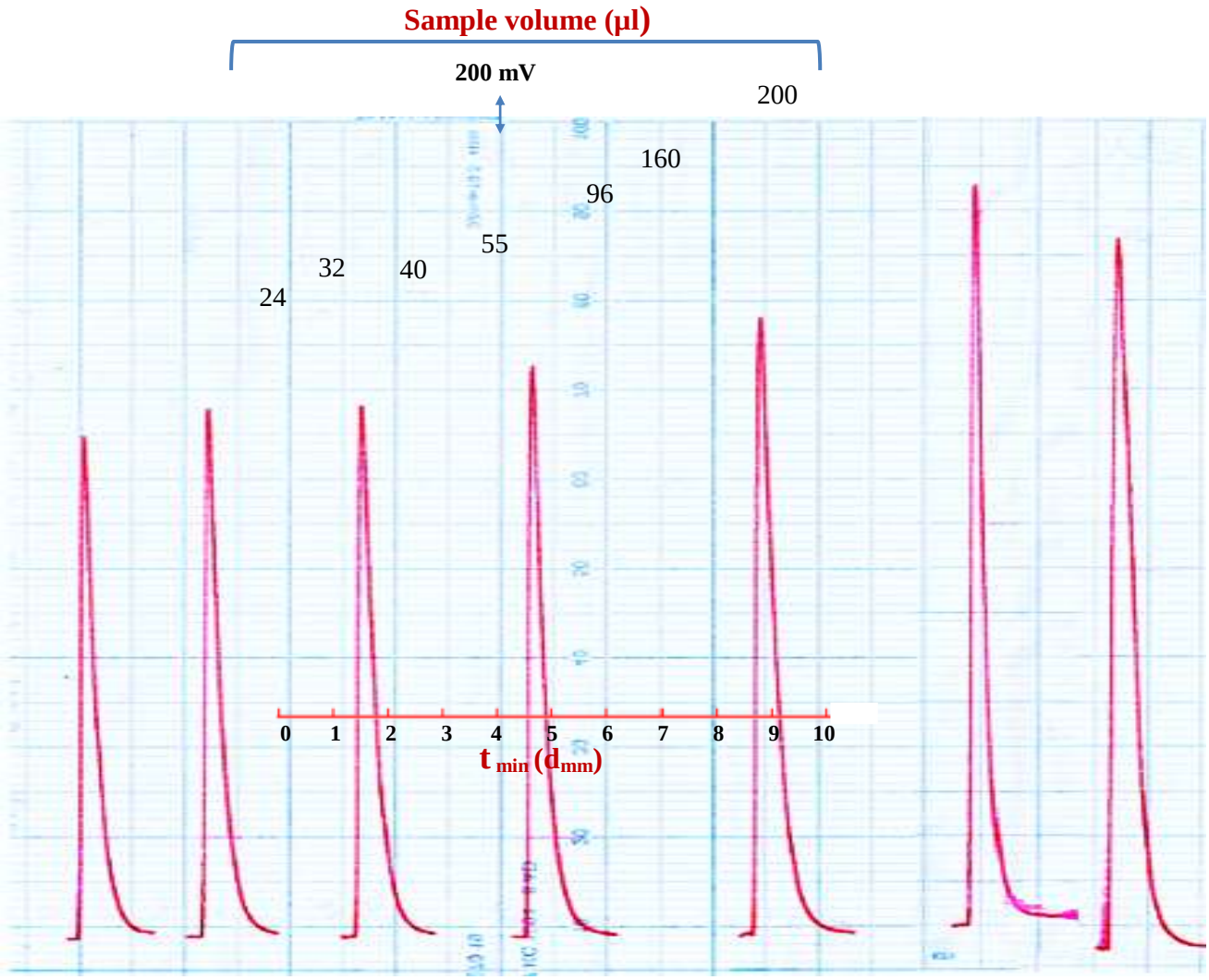


Fig. 7.A: Variation of sample volume on: Response profile $\bar{Y}Z_i$(mV) – t min (dmm)

Table 5: Segmentation pattern slope - intercept to select the optimum segment of sample plug (i.e.; a_5-a_7)

No. of segment	Sample volume (µL)	Segment	Slope mV/mmol.L^{-1}	Intercept mV
1	24-40	a_1-a_3	10.00	2600
2	23-55	a_2-a_4	10.97	2581
3	40-96	a_3-a_5	8.10	2697
4	55-160	a_4- a_6	9.30	2642
5	96-200	a_5- a_7	5.73	3000

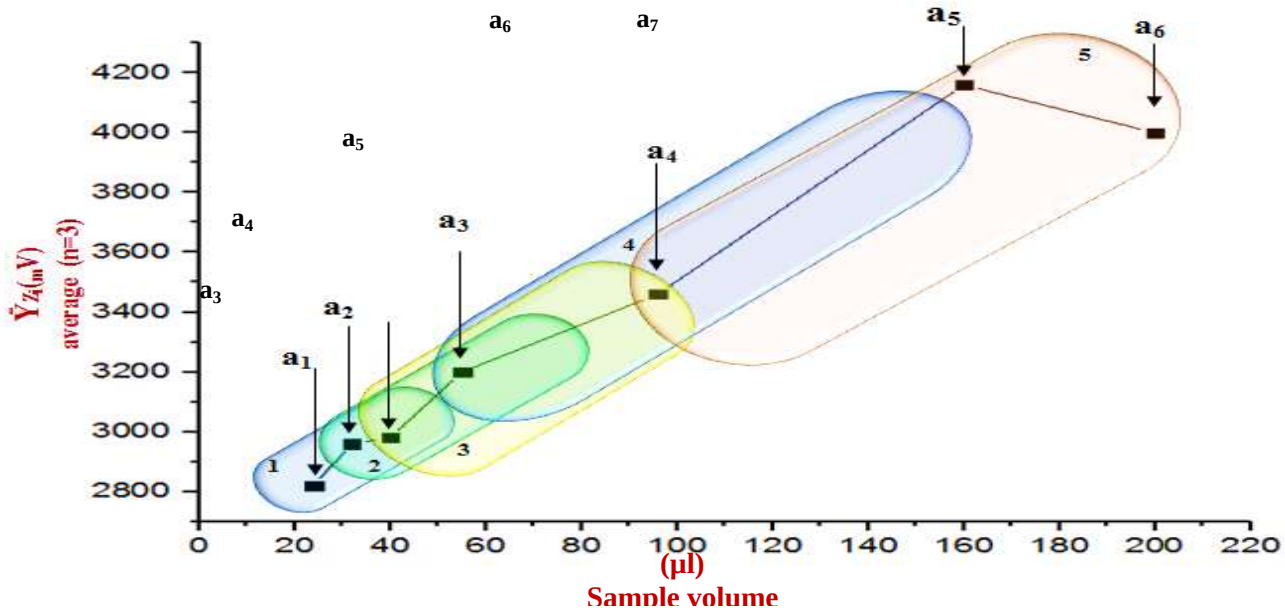


Figure 7 B. Five segments(Three point data) and chosen segments

Effect of Delay Reaction Coil (Teflon) for Completion of Reaction

This study was conducted on the STP(5mmol.L^{-1})-PMA(2mmol.l^{-1})- NaCl (70 mmol.L^{-1}) reaction system which was attached after Y- junction point (Fig.2).The volume of each reaction coil tabulated in Table 6.A .It was noticed that an increase of coil length causes a decrease in sensitivity in general ,this might be explained to the formation of larger particles, increase particulate weight and spreading it on a wider surface area which in turn to lead a difficulty in moving through flow cell. So, delayed reaction coils were avoided for use. This results corresponding with slope - intercept calculation (Table 6.B and Fig.8.), in which that the chosen of segment can be selected is a_2-a_4 (i.e.; 157- 314 µL).The 157 µL delay coils is within the chosen segment.

Table 6.A: Effect of the variation of coil length on $\bar{Y}Z_i$(mV):(S/N) energy transducer response in mV using STP(5mmol.L^{-1})- PMA(2mmol.l^{-1})-NaCl (70mmol.L^{-1}) system and 160µL sample volume.

Coil Length Cm r=0.5 (mm)	Coil Volume (µl)	$\bar{Y}_{Zi(mV)}$ average (n=3)	RSD %	Confidence nterval at 95% $\bar{Y}_{z\ i}$ (mV) ± $t_{0.05/2\ ,n-1}\ \sigma_{\ n-1}/\sqrt{n}$	t* sec	Base width Δt_B (sec)	V_{add} (ml) At flow cell	Concentration (mmol.L^{-1}) At flow cell
0*	0*	4160	0.026	4160 ± 2.757	16	84	5.90	0.136
20	157.0	4500	0.027	4500 ± 3.0557	17	86	6.04	0.133
30	235.5	3880	0.059	3880 ± 5.763	20	90	6.31	0.127
40	314.0	3840	0.067	3840 ± 6.409	24	99	6.93	0.115
50	392.5	3780	0.077	3780 ± 7.254	25	100	7.00	0.114

0*: Without coil, $\bar{Y}Zi\ (_mV)$: S/N) energy transducer response, Δt_B:Base with of response (sec), $t_{0.05/2,2}$=4.303

Table 6.B: Segmentation pattern to study, the effect of delay reaction coil

No. of segment	Sample volume (µL)	Segment	Slope mV/mmol.L^{-1}	Intercept mV
1	0-235.5	a_1-a_3	-70.97	4272
2	157.0-314.0	a_2-a_4	-4.20	5063
3	235.5-392.5	a_3-a_5	-63.69	4000

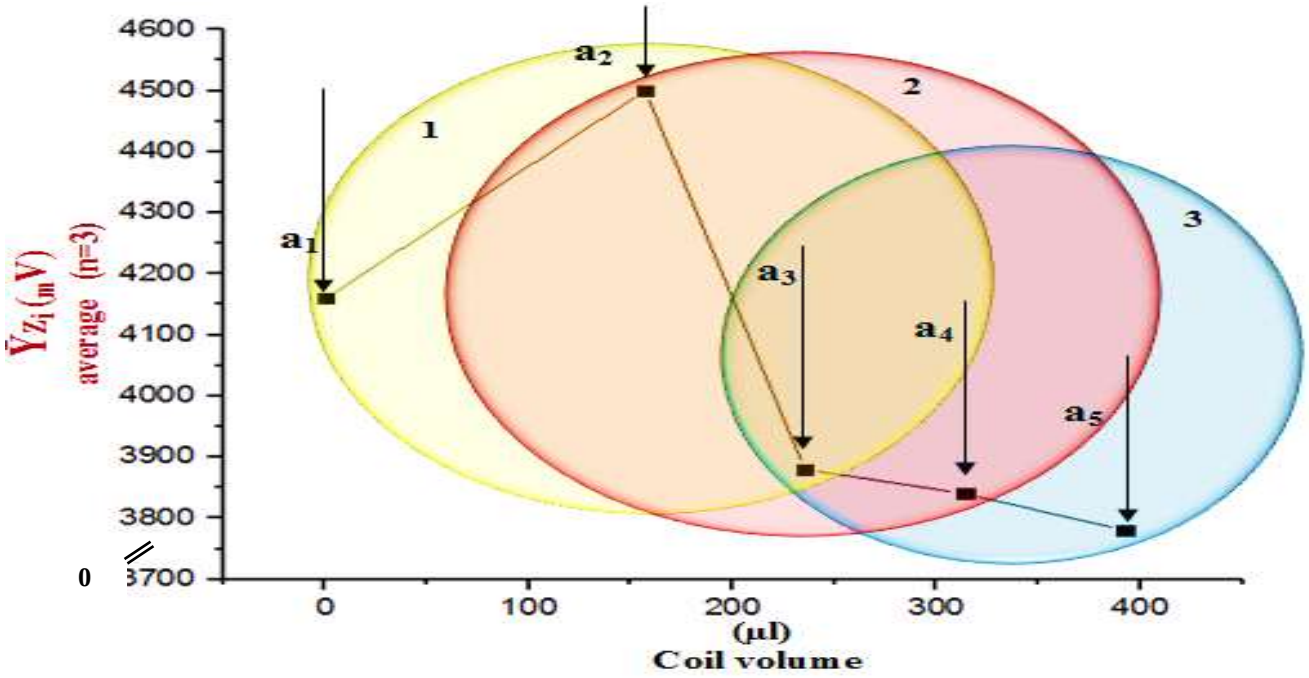


Fig.8: three data point as one segment and choice

Effect of Electronic Filter (Low Band Pass Electronic Noise Filter)

Using all optimum conditions concerning chemical and physical parameters for two line manifold design system, the effect of electronic filter variables on STP(5mmol.L^{-1})-PMA(2mmol.l^{-1})-NaCl (70mmol.L^{-1}) system were studied. Electronic filters with a time constant ranging from 0.1632 to 3.7740 sec were used to overcome the little tiny noise which developed by pulse of the pump. All this kind of electronic filter causing a decrease on the measured of sensitivity (Fig.9.A and B) .Therefor, electronic filter were a voided for use in this reaction system. And that was demonstrated by relying on slope- intercept calculation.Without electronic filter within the chosen segment (segment no.1 in table 7- part B and fig .9.B)was selected. The results of this study

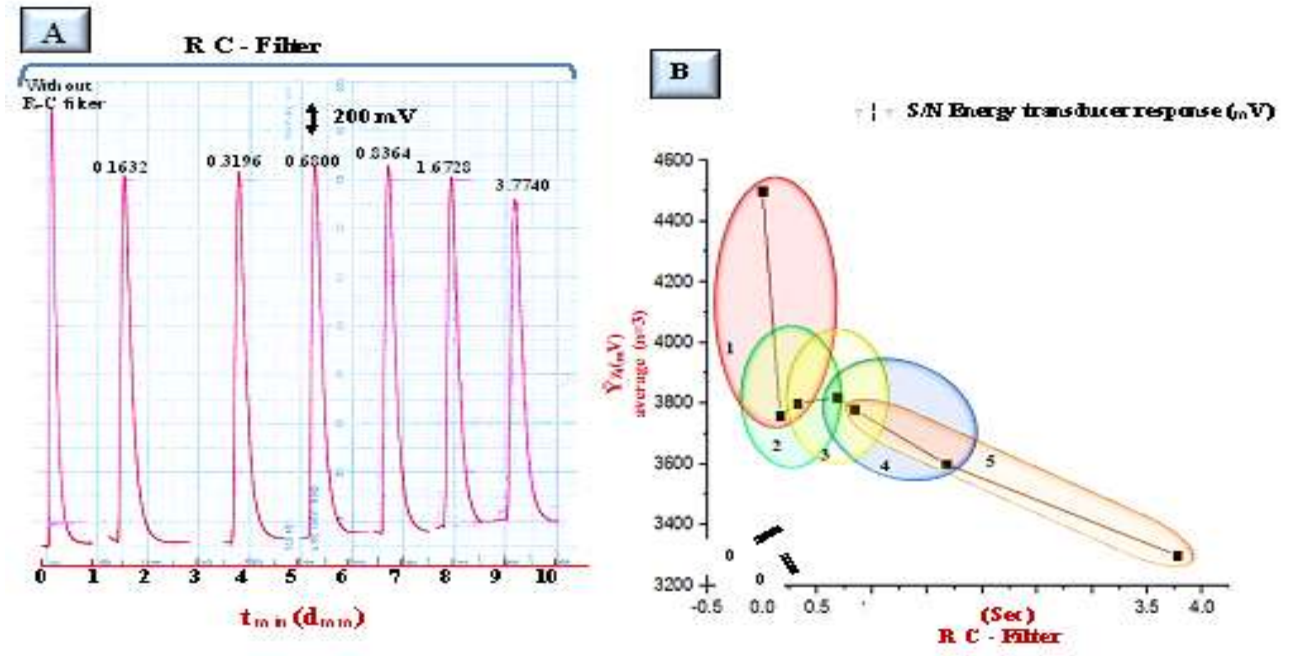


Fig. 9: Effect RC -Filter on:
A- Response profile $\bar{Y}Z_i$(mV)-t_{min} (d_{mm}).
B- Height of $\bar{Y}Z_i$(mV):(S/N) energy transducer response in mV with chosen segment average (n=3)and three data point as one segment and choice

Table 7: Effect of low band pass electronic filter on peak height of S/N energy transducer response

A			B: Three data point as one segment and choice			
R C-Filter (Sec)	**Confidence nterval at 95% $\bar{Y}z_i$ (mV) ± $t_{0.05/2,n-1}$ $\sigma_{n-1}/\sqrt{n}$**	**RSD%**	**No .of segment**	**RC-Filter (Sec)**	**Intercept mV**	**Slope mV/mmol.L^{-1}**
0*	4500 ± 3.055	0.027	1	0-0.3196	4375	-2207.71
0.1632	3760 ± 3.925	0.042	2	0.1632-0.6800	3752	105.53
0.3196	3800 ± 3.279	0.034				
0.6800	3820 ± 4.422	0.046	3	0.6800-1.6728	3814	-22.27
0.8364	3780 ± 3.676	0.039	4	0.6800-1.6728	3966	-219.47
1.6728	3600 ± 3.776	0.042				
3.7740	3300 ± 4.024	0.049	5	0.8364-3.7740	3894	-159.41

$\bar{Y}Z_i$ (mV): (S/N) energy transducer response, 0*: without filter, R C: Resistance x capacity

Estimating the Linear Dynamic Range from Scatter Plot for the Variation of STP on the S/N Energy Transducer Response

In previous section physical as well as chemical variable were set at their optimum values (STP-PMA (2 mmol.L^{-1})- NaCl (70 mmol.L^{-1}) system, 160µL sample volume, 157µL delay reaction coil and 2.0 ml.min^{-1}, 2.1 ml.min^{-1} flow rate for carrier stream and PMA line respectively, a set of series 0.1- 10 mmol.L^{-1} solutions were prepared.

A range 0.1-10 mmol.L^{-1} (i.e. choosing all sixteen points with correlation coefficient of **r = 0.8803** while analytical range explain , an increase of STP concentration causes an increase of precipitate particulats (i.e deals with directly proportional between STP concentration and S/N energy transducer response) up to 5.5 mmol.L^{-1} (n= 13 point) with **r= 0.9924** at range (0.1-5.5)mmol.L^{-1}.Above 5.5 mmol.L^{-1}, a broad in maxima of the peak height was obtained, this might be attributed to the an increase of precipitate particulats and its compactness thus leading to decrease of interstitial spaces and reflecting surface, in addition to an increase of particle size causing a slow movement of particles leading to a longer time duration of particles in front of the detector which in turn to obtain distorted response. Working range (0.1-4.7 mmol.L^{-1} (n=11)) with r=0.9961 and linear dynamic range (0.1-4.5 mmol.L^{-1} (n=10)).So, searching for better representation, a shorter range should be used to improve the assessment mathematical formation. The best fit linear equation representing the correlation between STP concentration (In dependent variable) against diverged light as dependent with has **r=0.9965** and % capital R- squared **of** 99.29% on the form:

$\hat{Y}z_i$(mV) =327.443 ±126.523+703.064±48.131[STP] mmol.L^{-1} was able to explain much of the obtained results from n=16 were the outcome of scatter plot. The assessment evaluation of the new developed methodology for the determination of STP was compared with available reference method [16] namely spectrophotometric method which is based on the measurements of absorbance for the variable range of concentration as shown in table 8 at λma =267 nm. The best linear range extend from 0.03-1.7 of n=10 with correlation coefficient of 0.9988 and % capital R-squared =99.77%.

Table 8: **Summary of different range regression for the variation of S/N energy transducer**

Type of mode	**Range of [STP] mmol.L^{-1}(n)**	**$\hat{Y}_i$=a±s_at+b±s_bt[STP]mmol.L^{-1} At confidence level 95 %, n-2**	**r , r^2, R^2 %**	**t_{tab} at 95 % ,n-2**	**Calculated t-value** $\frac{r/\sqrt{n-2}}{\sqrt{1-r^2}}$
Developed method using ISNAG-fluorimeter					
UV. Sp Classical method Absorbance measurement at λ_{max}=267 nm					
Scatter plot	0.1-10(16)	949.794±668.966+465.401±143.764[STP] mmol.L^{-1}	0.8803, 0.7750,		2.145<6.944

			77.50	
	0.03-4(14)	0.314±0.278+0.743±0.157[STP] mmol.L^{-1}	0.9477, 0.8981, 89.81	2.179<10.286
Dynamic range or analytical range	0.1-5.5(13)	238.945±211.613+768.681±63.050[STP] mmol.L^{-1}	0.9924, 0.9849, 98.49	2.201<26.834
	0.03-3(13)	0.190±0.150+0.895±0.102[STP] mmol.L^{-1}	0.9854, 0.9711, 97.11	2.201<19.226
Working range or calibration range	0.1-4.7(11)	305.129±138.389+721.210±48.065 [STP] mmol.L^{-1}	0.9961, 0.9922, 99.22	2.262<23.284
	0.03-2(11)	0.101±0.049+1.055±0.0461[STP] mmol.L^{-1}	0.9983, 0.9966, 99.66	2.262<51.577
Linear range or Liner dynamic range	0.1-4.5(10)	327.443 ±126.523+703.064±48.131[STP] mmol.L^{-1}	0.9965, 0.9929, 99.29	2.306<33.684
	0.03-1.7(10)	0.0902±0.039+1.083±0.042[STP] mmol.L^{-1}	0.9988, 0.977, 99.77	2.306<58.980

Response with sitagliptin phosphate concentration using first degree equation of linear $\hat{Y}=a+bx$ at optimum condition. $\hat{Y}i$: Estimated response(n=3) in mV for newly developed method and without unit for spectrophotometric for n=3 expressed as an average peaks heights of linear equation of the form $\hat{Y}=a+bx$ or absorbance value , r: Correlation coefficient,r^2:Coefficient of determination ,R^2:% capital R-sequare,R^2= explain variation / total variation,[STP] concentration of sitagliptin phosphate, sp: Spectrophotometry, Developed method:using ISNAG-fluorimeter, n:no.of measurements, $t_{tab.}$=0.05/2,n-2

Limit of Detection (LOD)

The limit of detection of STP were calculated using three different approach as tabulated in Table 9 as an injected sample volume of 160µL [17, 18].

Table 9: Ditection limit of STP using 160µL and three methods

Practically based on the gradual dilution for the minimum concentration in calibration curve		**Theoretical (slope method) based on the value of slope X=3S_B/slope**	**Theoretically (linear equation)based on the value of $\hat{Y}$= Y_b +3S_b**
Newly developed method (0.5)µmol.L^{-1}	Classical method (0.2)µmol.L^{-1}		
41.86 ng/sample	16.75 ng/sample	98.356 ng/sample	35.999µg/sample

X: value of LOD based on the slope, S_B: standard deviation of blank for 13 times, Y_b: average response for blank= intercept (a), S_b: standard deviation equal to $S_{y/x}$ (residual), newly developed method (ISNAG-fluorimeter), Classical methods (UV–spectrophotometric)

Repeatability

The repeatability and reality of the measurements of ISNAG-fluorimeter using resonance mercury lamp was studied at selected concentration of STP (1&4.5 mmol.L^{-1}).

A repeat of measurements for eight successive injection were measured and the obtained results are summed up in table 10 which shows , the minimum of the relative standard deviation (RSD%) less than 0.2 % (Fig.10) .

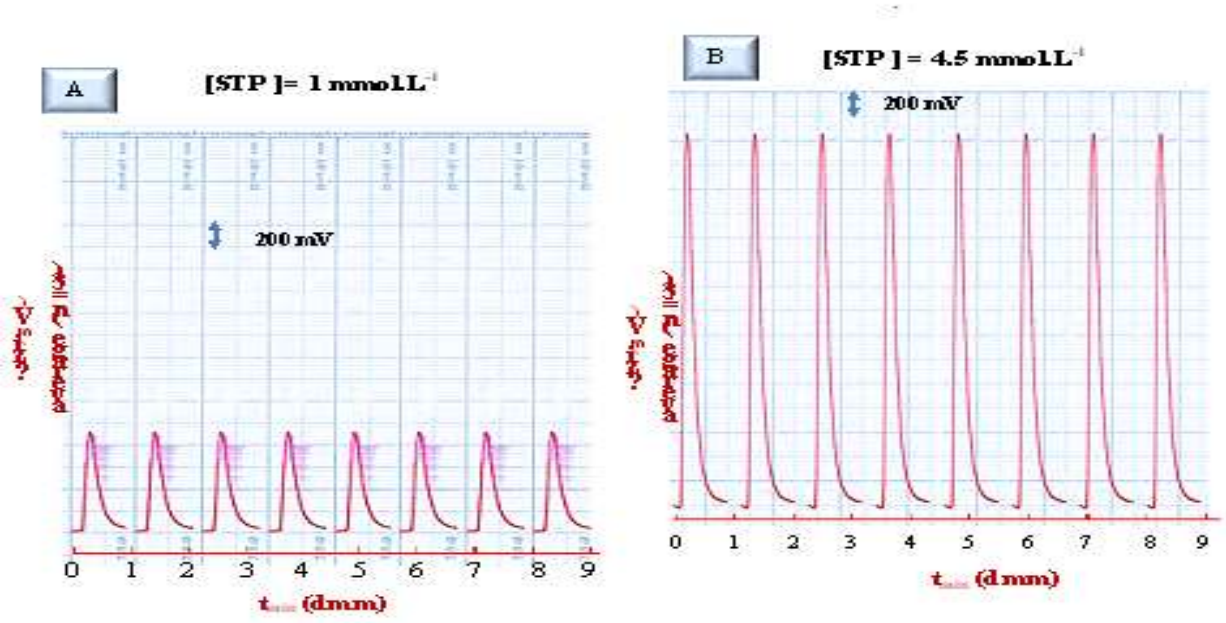


Fig.10: $\bar{Y}Z_i$ ($_m$V) – t_{min} (d $_{mm}$) profile of 8 successive measurement with a repeatability of profile for 1 and 4.5 mmol.L^{-1} concentration of STP.

Table 10: Repeatability results of STP at optimum parameters using 160µl sample volume and open valve mode.

[STP] mmol.L^{-1}	$\bar{Y}Z_i$ (mV) average (n=8)	RSD %	Confidence interval 95% at $\bar{Y}_{Z_i}$(mV)± $t_{0.05/2,7}\ \sigma_{n-1}/\sqrt{n}$
1.0	1120	0.164	1120±1.543
4.5	3580	0.065	3580±1.960

$\bar{Y}_{Zi\ (mV)}$:S/N as an mV energy transducer response expressed, $t_{0.05/2,7}$=2.365 , n= number of injection

Application of the use of ISNAG-fluorimeter for the Determination of STP in the different Drugs

The newly developed methodology was used for the determination of STP in two different drugs from different companies (sitavia-pioneer-Iraq (100mg) and Januvia-MSD-UK (100mg).Using STP–PMA (2mmol.L^{-1})–NaCl(70mmol.L^{-1}) system and was compared with uv-spectrophotometric (uv-1800 shimadzu) via the measurement of absorbance at λ max =267 nm [16].

The standard addition method for both methods were applied by preparing a series of solutions from each pharmaceutical drug via transferring 1.25 ml of each sample (10 mmol.L^{-1}) to five volumetric flasks (25 ml), followed by the addition of (0, 0.25, 0.35, 0.45 and 0.5 ml) from 50 mmol.L^{-1} standard solution of STP in order to have the concentration range from 0-1mmol .L^{-1}.

The results were tabulated and mathematically treated in table 11.A using newly developed method and classical uv-spectrophotometry at 95% confidence level (α =0.05/2 two tailed) .While table 11.B shows a practical content of active ingredient and recovery % in addition to a comparison between two methods. A comparative study will be conducted to establish the most suitable method and sensitive for the determination of STP in different drugs, and if there is a significant difference between two different drugs or two methods.

Two modes of comparison will be carried out that will be depends on the results tabulated in the Table 11.B One mode: Individual t-test between practically of active ingredient and claimed value (i.e; µ=100mg)

A hypothesis can be estimated as follow:

Null hypothesis: There is no significant difference between the means of weight (wi) with claimed value (µ) of officially British pharmacopeia i.e.; [20] :

H_o: $\mu_{100mg} = \overline{w_i}$ (for each different company)

Against, Alternative hypothesis: there is a significant difference in the amount of STP of the different companies with claimed value i.e.

H_1: $\mu_{100mg} \neq \overline{w_i}$ (for each different company).

Since all value obtained are t_{tab} ($t_{0.05/2,n-1}$) >> t_{cal} at α = 0.05, the decision is to accept the null hypothesis (i.e.; that there is no significant difference in the means with a specific population mean (µ= 100mg) and will be reject the alternative hypothesis.

Two mode: paired t-test assum that variation of different manufacturing companies does not affect on the content of available active ingredient in each sample. The obtained results as shown in table 11.B; in which a hypothesis can be estimated as follow:

Null hypothesis: There is no significant difference between the means obtained in all measured data.

i.e.; H_o: $\mu_{ISNAG\text{-}fluorimeter} = \mu_{uv\text{-}spectrophotometry}$

Against

Alternative hypothesis: There is a significant difference between two methods

i.e.; H_1: $\mu_{ISNAG\text{-}fluorimeter} \neq \mu_{uv\text{-}spectrophotometry}$

Since, the value of t_{cal} / -1.752/< t_{tab} (12.706); null hypothesis will be accepted and will be rejected the alternative hypothesis. These mean that there is no significant difference between the measurements of the two methods. And we can use anyone from methods for the analysis of STP in different samples. In addition to the make use of the advantages of using F-test to predict which of the methods is more precise than other method. Results from table 11.B column 7 that the developed new achieved method is much more precise than usual classical method (reported in the cited literature [16]. Since, variation of newly developed method (S_1= 0.0145) less than variation of classical method (S_2) =5.216.

Table 11: A: Summary of results for variable sample from different companies for determination of STP using: STP- PMA (2mmol.L^{-1}) 25ml – NaCl (70mmol.L^{-1}.) system, sample volume 160µL

No. of sample	Commerial Name, Company Content Country	Type of method: Newly developed method using ISNAG-fluorimeter (mV) UV. Sp Classical method Absorbance measurement at λ_{max}=267 nm: Confidence interval For the averge Weight of Table $\bar{w}i \pm 1.96\sigma n-1/\sqrt{n}$ at 95% (g)	Weight of Sample equivalent to 0.523 gm (10 mmol.L^{-1}) Of the active Ingredient w	Theoretical content for the active ingredient at 95% (mg) Wi±1.96σn-1 /√n	Sitagliptine [mmol.L^{-1}] for newly developed& Classical methods					Equation of standard addition at 95% for n-2	r / r^2 / R^2%
					0	0.25ml	0.35ml	0.45ml	0.5ml	$\hat{Y}i_{(mV)}=a\pm s_a t+b\pm s_b t$ [STP]mmol.L^{-1}	
					0	0.5	0.7	0.9	1	$\hat{Y}i=a\pm s_a t+b\pm s_b t$ [STP]mmol.L^{-1}	
1	Sitavia STG=100mg Pioneer	0.5615±0.0046	2.9384	100±0.8281	730	1400	1680	2020	2180	705.7643±102.2904+1445.5411±143.2351 [STP]mmol.L^{-1}	0.9985 / 0.9971 / 99.71

	Iraq				0.521	1.012	1.271	1.411	1.582	0.5143±0.1004+1.0405±0.1406 [STP]mmol.L^{-1}	0.9973 0.9946 99.46
2	Januvia STG=100mg MSD UK	0.4132±0.0013	2.1623	100±0.3202	720	1300	1580	1940	2100	673.8216±185.0075+1377.7070±259.0622 [STP]mmol.L^{-1}	0.9948 0.9896 98.96
					0.601	1.213	1.423	1.635	1.815	0.6037±0.0795+1.1834±0.1114 [STP]mmol.L^{-1}	0.9987 0.9973 99.73

Ȳi: Energy transducer in mV for developed method and absorbance for UV-Spectrophotometer method, r: correlation coefficient, r^2 coefficient of determination,R^2%:Percentage capital R- squared:R^2= explain variation as a percentage / total variation ,UV- Sp: UV– spectrophotometric method, $t_{0.05/2,\infty}$ =1.96 at 95 %, $t_{0.05/2,3}$ = 3.182 for n=5

Table 11. B:summary of results for two mode of comparison: Individual t-test (between claimed value and practically value of STP) and paird t-test (between two methods)

No. of sample	Type of method					F-test
	Developed method using ISNAG-Fluorimeter (mV)					
	UV. Sp Classical method Absorbance measurement at λ_{max}=267 nm					
	Practical concentration (mmol.L^{-1}) in 25 ml ---------- Practical concentration	Weight of STG in each sample (g) $W_{\overline{i(g)}} \pm 4.303\sigma n\text{-}1/\sqrt{n}$	Efficiency of determination Rec.%	Individual t-test For compared between claim value & practical value ($W_{\overline{i(mg)}}$-μ) √ n /σn-1	Paired t –test Compared between two methods	

	(mmol.L^{-1}) in 100 ml --- Practical weight of STG in (g)	Weight of STG in tablet $w_{i(mg)}$ ±4.303σn-1/√n			t_{cal}= Wd√n/σn-1	tab at 95% confidence level (n-1)	S^2 2 F_{cal}= —— S^2 1	F_{tab}
1	0.488 --- 9.765 --- 0.511	0.511 ±0.073 97.650±13.950	97.65	/-0.725 / <4.303	/ -1.752/ <12.706		359.76>>161.4	
	0.494 --- 9.885 --- 0.517	0.517±0.045 98.850±8.599	98.85	/-0.600 / <<4.303				
2	0.489 --- 9.782 --- 0.512	0.512±0.056 97.820±10.70	97.82	/-0.877/<<4.303				
	0.5101 --- 10.203 --- 0.534	0.534±0.083 102.030±15.860	102.03	0.550 <<4.303				

μ: claim value (mg) =100 mg, Wi: practically weight (n=3),Wd: average of different between two methods (developed & classical), t_{tab} =$t_{0.025,1}$=12.706 for paired t-test, n(no.of samples) =2 , σ_{n-1}: difference of standard deviation, $t_{0.025,2}$=4.303 (for Individual t-test), F_{tab}=$F_{0.95,\upsilon1,\upsilon2}$,=$F_{0.95,1,1}$=161.4.

Conclusion

The newly developed method for the determination of sitagliptin phosphate in pharmaceutical preparations (drugs) is simple, sensitive and rapid. this method based on precipitation sitagliptin phosphate (STP) via PMA (as precipitating agent), this formed precipitate will be irradiated by two main wavelength using low pressure mercury lamp at 184.9nm and 253.7 nm and measument the scatared light at 90^0 using ISNAG-fluorimeter analyser. An alternative analytical method is found through this research work which was based on simple parameter conditions.

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