INTERNATIONAL JOURNAL OF RESEARCH IN PHARMACY AND CHEMISTRY

Available online at www.ijrpc.com

Research Article

QUENCHING OF FLUORESCENCE FOR DETERMINATION OF OXONIUM ION USING LASER DIODE FLUORIMETER: (BLUE PURPLE(405 NM)-GREEN (532NM) VIA FLUORESCEIN SALT AS A FLUOROPHORE MOLECULE AT 405NM AND I^--IO_3^--H_3O^+ SYSTEM

Nagam STurkey Al-Awadie[1*], and Marwa A Kadhim Al-banaa[2]

[1]Department of chemistry, College of science, University of Baghdad, Baghdad, Iraq.
[2]Department of optics techniques, Dijlah Collage University, Baghdad, Iraq.

ABSTRACT

A simple, rapid and sensitive method for the determination of oxoniumion in strongly inorganic acid (HCl, H_2SO_4 and $HClO_4$), and organic acid (tartaric acid) were developed by continuous flow injection analysis coupled with laser diode fluorimeter. The method is based on the generation of iodine molecule from the I^--IO3$^-$ -H_3O^+reaction; the liberated iodine is then reacted with fluorescein salt solution causing to quench the fluorescence light (continuous fluorescence) when irradiated by laser source at 405nm.A linear range of HCl, H_2SO_4, $HClO_4$ and tartaric acid respectively was 0.07-3, 0.07-3, 0.05-7 and 0.1-7mMol.L^{-1}, with correlation coefficient r=0.9904, 0.9609, 0.9527 and 0.9782, limit of detection (LOD) 0.09, 0.26.0.27 and 0.41ng/sample based on gradual dilution of lowest concentration in calibration graph. The percentage relative standard deviation for 0.9mMol.L^{-1} (HCl) , 0.3mMol.L^{-1}(H_2SO_4), 1mMol.L^{-1} ($HClO_4$) and 2mMol.L^{-1}(tartaric acid) is lower than 1% (n=8).A comparison was made between the newly developed method of analysis with the classical method (pH-meter) using the standard addition method via the use of paired t-test. It shows that there was no significant difference via the use of t-test at α=0.05 (95% confidence) between either methods. On this basis the new method can be accepted as an alternative analytical method for determination of oxonium ion in commercial samples.

Keywords: Oxonium ion, Laser diode fluorimeter, Flow injection analysis, Fluorescence.

1. INTRODUCTION

Acids a compounds usually having a sour taste and capable of neutralizing alkalis and reddening blue litmus paper, containing hydrogen that can replaced by a metal or an electropositive group to form a salt, or containing an atom that can accept a pair of electrons from a base. Acids are proton donors that yield hydronium ions in water solution, or electron-pair acceptors that combine with electron-pair donors or bases[1-3]. Common examples of acids include hydrochloric acid(a solution of hydrogen chloride which is found in gastric acid in the stomach and activates digestive enzymes), sulfuric acid (used in car batteries), perchloric acid, it is an oxidizing agent, and tartaric acid (a solid used in baking)[4,5]. Some of the most commonly used methods for determination of acid (H_3O^+) include atomic absorption spectrophotometry[6], spectrophotometry[7],potentiometric titration[8], flow injection analysis[9-12] and chemilumenescence[13,14].Fluorescence is a member of the ubiquitous luminescence family of processes in which susceptible molecules emit light from electronically excited states. Generation of luminescence through excitation of a molecule by ultraviolet or visible light photon is a phenomenon termed photoluminescence which is formally divided into two categories, fluorescence and phosphorescence depending upon the electronic configuration of the excited state and the emission pathway. Fluorescence is the property of some atoms and molecules to absorb light at a particular wavelength and to subsequently emit light of longer wavelength after a brief interval, termed the fluorescence lifetime[15,16].This method combined with flow injection analysis technique and has been used for determination of oxonium ion via the fluorescence quenching system I^--IO_3^--H_3O^+ using fluorescein salt as a fluorophore molecule.

2. Experimental

2.1Reagents and chemicals

A stock solution (0.01 Mol.L^{-1}) of fluorescein salt ($C_{20}H_{10}Na_2O_5$,M.Wt376.27 g.moL^{-1} , Hopkin&William) was prepared by dissolving 1.8813g in 500 ml of distilled water . A stock solution of potassium iodide (KI ,M.Wt 166 g.mol^{-1} ,BDH,0.3Mol.L^{-1}) was prepared by dissolving 24.90g in 500mL of distilled water and potassium iodate (KIO_3, M.Wt214g.mol^{-1}, BDH,0.25Mol.L^{-1}) was prepared by dissolving 26.7500g in 500mL of distilled water. A stock solutions of acids (hydrochloric acid (38% w/w , 1.19 g.ml^{-1},BDH,2Mol.L^{-1}), sulphuric acid (98% w/w , 1.84 g.ml^{-1}, BDH,2Mol.L^{-1}) and perchloric acid (85% w/w , 1.69 g.ml^{-1},BDH,2Mol.L^{-1}) was prepared by pipetting 161.43mL, 108.78mL and 139.86mL respectively of concentrated acids and complete the volume with distilled water to 1000mL volumetric flasks. Each acid was standardized against standard solution of 2Mol.L^{-1} from Na_2CO_3 (BDH ,105.99 g.mol^{-1}) ;which prepared by dissolving 21.1980 g in 100 ml distilled water . A stock solution of tartaric acid ($C_4H_6O_6$,M.Wt 150.09 g.mol^{-1} ,BDH, 0.1Mol.L^{-1}) was prepared by dissolving 1.5009g in 100mL of distilled water.

2.2 Apparatus

Laser diode fluorimeter is a homemade instrument that is capable in measuring fluorescence light at two available laser diodes having the wavelength at 405nm (10mW) & 532nm laser diode of not less than 1000mW. Each radiation source is fitted with a 2mm flow cell in a block of brass metal equipped with a photo diode detector. The angle between the radiation source at an aperture of 2mm as a maximum radiation area for a flow cell having outside diameter ,4mm inside diameter 2mm (path length for irradiation). The angle between irradiation source-flow cell- detector is 90^o .The whole instrument composed of five main parts which are as follows :fluorescence cell(composed of cubic (50mm (L), 50mm (W), 50mm (D)) brass metal block) , flow cell(quartz silica having the length of 60mm), detector(photo diode having the diameter of a 4mm which respond to the visible area) , irradiation sources(two laser sources have been used. The first source blue-violet having the wavelength 405nm it's a solid state laser of continuous wave with a light intensity equivalent to 1800-2000Lux at a distance of 1mm (distance of the source to the detector). Second source green it's a solid state laser with a continuous wave of 532nm with a light intensity more than 2000Lux), and general panel of instrument.All tubes are made of Teflon 1mm inside diameter 2mm outside. Peristaltic pump – 1 channel variables speed (Ismatec , Switzerland)and a rotary 6-port injection valve(IDEX corporation ,USA) with a sample loop (0.5mm id, Teflon, variable length) used for sample injection. The output signals was recorded by x-t potentiometric recorder (KOMPENSO GRAPH C-1032) Siemens (Germany).

2.3 Methodology

The flow diagram of the whole reaction manifold system used for the determination of oxonium ion via the fluorescence quenching system I^- -IO_3^- - H_3O^+ usinguranine (fluorescein salt) as a fluorophore molecule is shown in fig.1. The manifold system is composed of one line (0.5 mMol.L^{-1} fluorescein solution) at 1.30mL/min flow rate which leads to the injection valve no.$_1$ to carry oxonium ion as a sample segment (2mMol.L^{-1}HCl), 27µL while the second injection valve load with mixing solution (1mMol.L^{-1} I^-& 0.5mMol.L^{-1} IO_3^-) ,31µL. Both segment samples meet together when injection valve no.$_1$ and valve no.$_2$opened sequentially and then the liberated iodine will quench the fluorescence of fluorescein sodium salt molecule followed, the segment pass through flow measuring cell. The response profile of which was recorded on x-t potentiometric recorder to measure quenching of fluorescence expressed as peak height in mV.

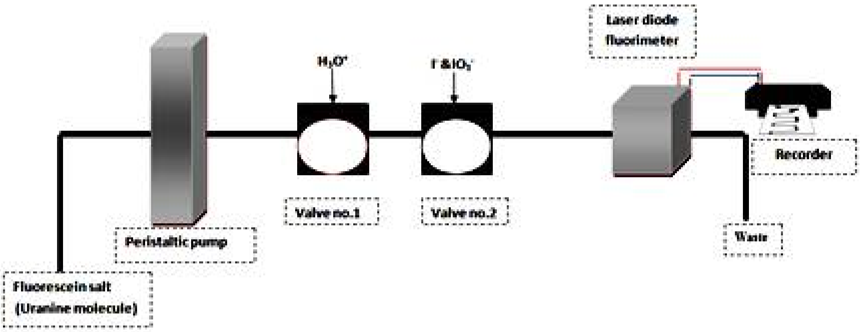
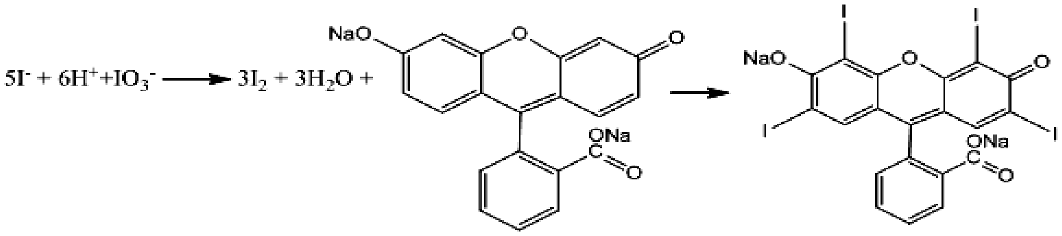


Fig.1: Schematic diagram of continuous flow injection analysis-Laser diode fluorimeter using one line manifold system

The proposed suggested mechanism for the formation of derivative of fluorescein molecule with iodine (i.e. Erythrosine)[15]according to the following scheme 1.



Fluorescein salt **Erythrosine $C_{20}H_6I_4Na_2O_5$**

Scheme 1: Proposed mechanism of the reaction between fluorescein and I_2

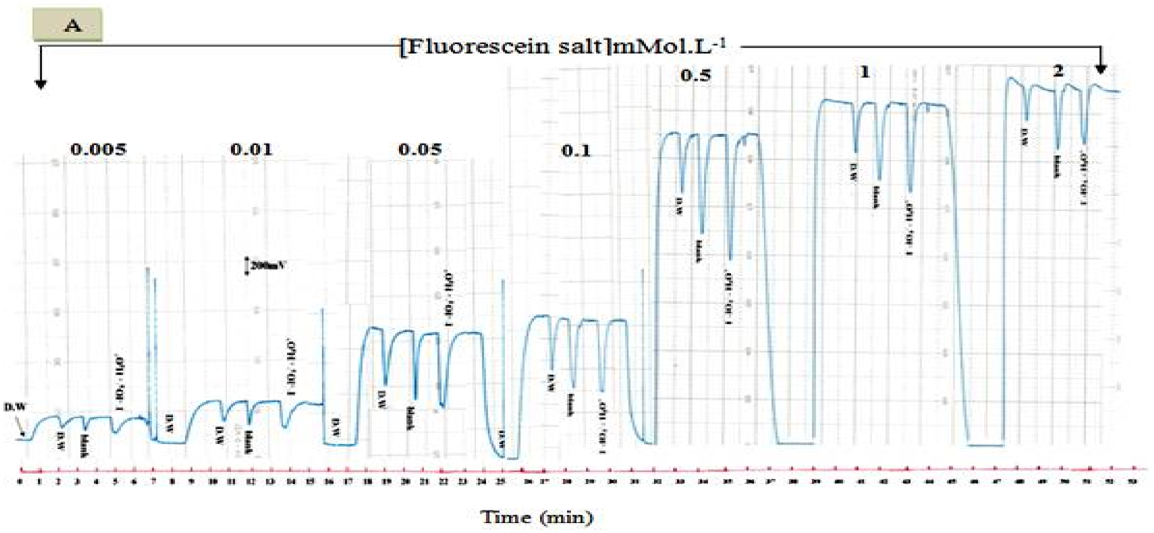
3.1Variable optimization

Chemical and physical parameters using one manifold system (fig.1.A) were studied in order to obtain the optimum conditions for the determination of oxonium ion.

3.1.1 Chemical parameters

3.1.1.1. Fluorescein salt concentration

A series of solutions having the concentration, ranging from 0.005-2mMol.L^{-1} were prepared. These prepared solution were used to study the effect on the intensity of fluorescence for steady constant fluorescence and continuous. At the same time the following injected sample were made : -Injection of H_2O in valve no.$_2$ (39µL) &closed valve no.$_1$-Injection of I^- (1mMol.L^{-1}) & IO_3^- (0.1mMol.L^{-1}) in valve no.$_2$ & closed valve no.$_1$ Followed by : injection of H_3O^+ (2mMol.L^{-1} of HCl) in valve no.$_1$ (35µL) and 39µL from I^-& IO_3^- in valve no.$_2$ at flow rate 1.30mL/min and using sequential open valve . The response profile shows in fig.2.A and tabulated in table 1. Obtained results showed that increase in fluorescence with increasing fluorescein also increased quenching effect using of I^- - IO_3^- -H_3O^+ system. This was the case up to 0.5mMol.L^{-1}. Above this concentration; it was noticed that a decreased quenching effect by I_2 due to large increase in fluorescent molecules. It is on this basis and in order to compromise between increased fluorescence and sensitivity of H_3O^+ ion determination through obtaining on higher quenching in terms of obtaining negative response; 0.5mMol.L^{-1} was chosen as the optimum concentration (fig.2.B).



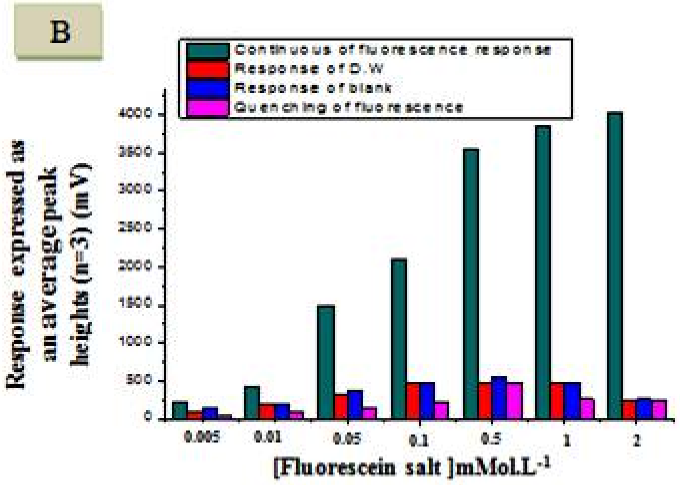


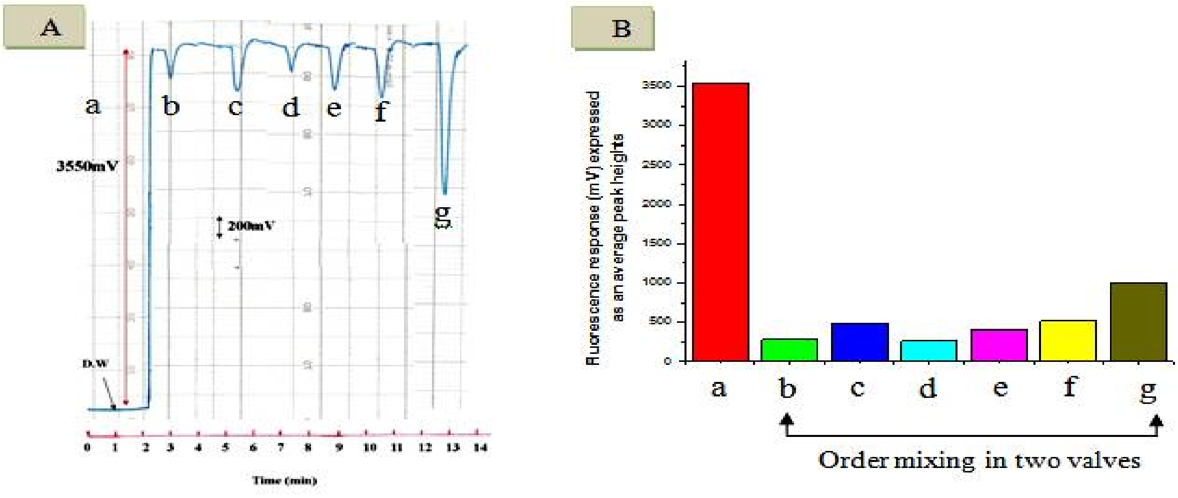
Fig. 2: Variation of fluorescein salt concentration (mMol.L^{-1}) on: A-Response profile B-Response height of continuous fluorescence, quenching of fluorescenceby: D.W, blank (I^--IO_3^-) and I^--IO_3^--H_3O^+ system

Table 1: Effect of variation of fluorescein salt concentration on quenching by: D.W, I^- &IO_3^- (blank) & I^--IO_3^--H_3O^+ system

Concentration of fluorescein salt mMol.L^{-1}	Continuous of fluorescence response (mV)	Quenching response by D.W (mV)	Quenching response by blank (mV)	Total quenched fluorescence expressed as an average peak heights(n=3)$\bar{y}_i$ in mV	Quenched fluorescence $\bar{y}_{Qi}$ (n=3)mV	Remained fluorescence $\bar{y}_{Ri}$(n=3)mV
	$\bar{y}_i$(mV)$\pm t_{0.05/2,\,n-1}\ \sigma_{n-1}/\sqrt{n}$					
0.005	220±0.0	100±2.48	140±2.48	180±1.9	40	40
0.01	420±1.73	200±2.00	200±0.49	280±1.47	80	140
0.05	1500±1.49	320±4.70	380±4.97	520±1.69	140	980
0.1	2100±1.24	460± 1.34	470±1.24	700±2.87	230	1400
0.5	3550±0.49	480± 4.55	540±1.74	1010±1.32	470	2540
1	3840±0.74	460±4.97	480±0.75	740±4.3	260	3100
2	4020±0.99	240±1.24	260±0.49	500±3.31	240	3520

3.1.1.2. Chemical reactants effects & order of mixing in valves

A set of experiment were carried out in order to establish the effect of chemical reactants with differences in using either/or both valves . Using the optimum concentration of fluorescein salt solution (0.5mMol.L^{-1}) as a carrier stream while 35µL sample volume injected at valve no.$_1$ (2 mMol.L^{-1}HCl) and 39µL injected at valve no.$_2$(1mMol.L^{-1} of I^- & 0.1Mol.L^{-1} of IO_3^-) at flow rate 1.30mL/min using sequential open valves mode. The results are shown in fig.3 and tabulated in table 2.



A- Response profile
B-a: Total continuous response of fluorescence . Quenchingby :
b:Close valve no.$_1$ and open valve no.$_2$ (D.W)
c : Open both valves (D.W)
d:Open valve no.$_1$(HCl) and close valve no.$_2$
e:Open valve no.$_1$(HCl) and open valve no.$_2$ (D.W)
f:Close valve no.$_1$ and open valve no.$_2$ (I^--IO_3^- blank)
g:Open valve no.$_1$(HCl) and open valve no.$_2$ (I^--IO_3^-)

Table 2: Effect of the chemical reactants and order of mixing in valves on the quenching of continuous fluorescence

Differences in chemical reactants and order mixing in two valves	Quenching response expressed as an average peak heights (n=3)$\bar{y}_i$ in mV	RSD%	Confidence interval of the average response (95% confidence level) $\bar{y}_i$(mV)±$t_{0.05/2,\,n-1}$ $\sigma_{n-1}/\sqrt{n}$	Percentage effect
Close valve no.$_1$ and open valve no.$_2$ (D.W) (b)	280	0.07	280±0.49	7.89
Open both valves (D.W) (c)	480	0.08	480±0.95	13.52
Open valve no.$_1$ (HCl) and close valve no.$_2$(d)	260	0.19	260±1.23	7.32
Open valve no.$_1$(HCl) and open valve no.$_2$ (D.W) (e)	400	0.37	400±3.68	11.27
Close valve no.$_1$ and open valve no.$_2$ (I^- -IO_3^- blank) (f)	520	0.19	520±2.45	14.65
Open valve no.$_1$ (HCl) and open valve no.$_2$ (I^- -IO_3^-) (g)	1000	0.09	1000±2.24	28.17

Response of continuous fluorescence : 3550mV

A conclusion can be drawn from this study; that the first valve should be used for sample introduction while the second valve is used for charging the reactants. This suggested mode of working was found to be the best to obtain 28.17% quenching effect.

3.1.1.3. Variation of iodide and iodate ions concentration

A series of iodide ion solutions having the concentrations of 0.01-10mMol.L^{-1} at constant concentration of iodate ion 0.1mMol.L^{-1} was prepared using 39µL as an injected at valve no.$_2$ with preliminary concentration of oxonium ion (2mMol.L^{-1}HCl) at valve no.$_1$, 35µL sample volume, flow rate of carrier stream (fluorescein sodium salt solution 0.5mMol.L^{-1}), 1.30mL/min flow rate. Using sequential open valves mode. Fig.4. A$_1$ was obtained explaining the increase of iodide ion concentration leads to increase of quenching fluorescence response up to 1mMol.L^{-1} , while at higher concentration (more than 1mMol.L^{-1}) decrease in quenching fluorescence response therefore 1mMol.L^{-1} was chosen as optimal concentration of iodide ion (fig.4..B$_1$). Table 3 summarizes the results obtained. After selected the optimum concentration of iodide ion a series of iodate ion solutions (0.01-10 mMol.L^{-1}) were prepared. Fig.4.A$_2$ and table 3 summarizes the obtained results. It can be shows that 0.5mMol.L^{-1} (fig.4.B$_2$) was the optimum concentration of iodate ion which gave a highest quenching fluorescence response and lower remained of fluorescence intensity.

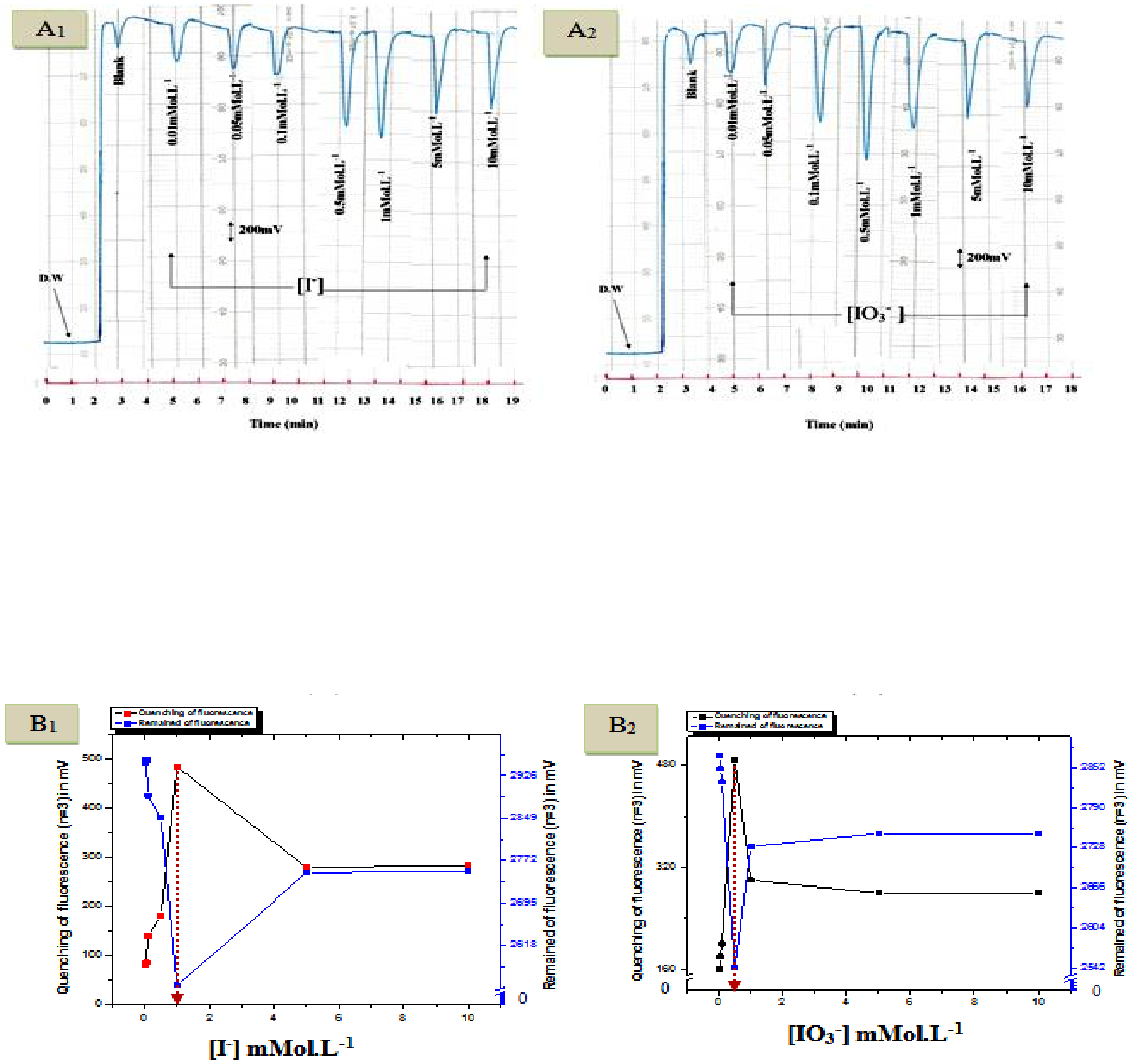


Fig. 4: Variation of iodide ion concentration and iodate ion concentration on the:A$_1$,A$_2$-Response profile using Laser Diode Fluorimeter.B$_1$,B$_2$-Quenching ofcontinuous fluorescence response and remained fluorescence response

Table 3: Variation of I^- and IO_3^- concentration on the total quenching of fluorescence expressedas an average peak heights (n=3)

Variation of iodide ion concentration					
concentration mMol.L^{-1}	Total quenched fluorescence expressed as an average peak heights (n=3) $\bar{y}_i$ in mV	RSD%	Confidence interval of the average response (95% confidence level) $\bar{y}_{i(mV)} \pm t_{0.05/2,\, n-1}\sigma_{n-1}/\sqrt{n}$	Quenched fluorescence $\bar{y}_{Qi}$ (n=3)mV	Remained fluorescence $\bar{y}_{Ri}$(n=3)mV
0.01	600	0.13	600±1.94	80	2950
0.05	600	0.01	600±0.15	80	2950
0.1	660	0.02	660±0.33	140	2890
0.5	700	0.33	700±5.74	180	2850
1	1005	0.04	1005±0.99	485	2545
5	800	0.17	800±3.38	280	2750
10	800	0.38	800±7.55	280	2750
Variation of iodate ion concentration					
0.01	680	0.28	680±4.73	160	2870
0.05	700	0.16	700±2.78	180	2850
0.1	720	0.07	720±1.25	200	2830
0.5	1010	0.23	1010±5.77	490	2540
1	820	0.56	820±11.41	300	2730
5	800	0.23	800±4.57	280	2750
10	800	0.19	800±3.78	280	2750

Response of continuous fluorescence : 3550mV,Response of blank(I^-&IO_3^-) : 520mV

3.1.2 Physical parameter

3.1.2.1 Flow rate

A study was carried out for the determination of preffered flow rate within the range of 0.20 to 1.75mL/min using quenching reactants of 39µL from 1mMol.L^{-1} I^- and 0.5 mMol.L^{-1} IO_3^- injected at valve no.$_2$. It react with oxonium ion (35µL) having the concentration of 2mMol.L^{-1}HCl which where injected at valve no.$_1$ to formation iodine molecule that quench fluorescence (fluorescein salt as a carrier stream 0.5mMol.L^{-1}) when opened valve no.$_1$ and valve no.$_2$ sequentially. The total results obtained were tabulated in table 4. It was noticed that at low flow rate there is an increase in dilution effect and peak base width (Δt_b) of the response accompanied with a decrease in peak height while at high flow rate (> 1.30mL/min) there was a decrease in dilution effect (dilution factor) on the responses obtained and increase in peak height that was attributed to the decrease of the time required for the departure of sample segment from injection valve no.$_2$ to the measuring cell. Therefore a flow rate of 1.30mL/min was chosen as the optimum flow rate (fig.5)that can give a regular response and low dilution& dispersion effect on the segment of I_2 through the manifold system.

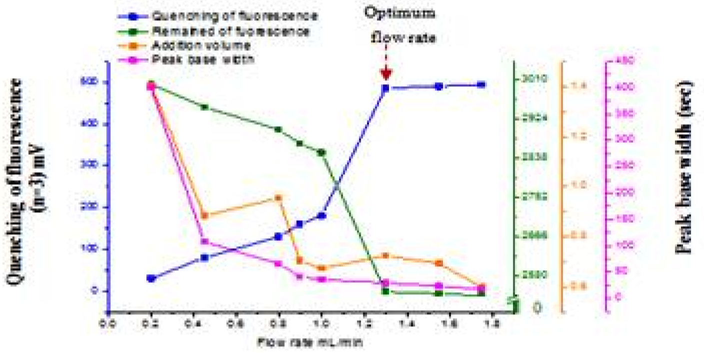


Fig. 5: Variation of flow rate on quenching of fluorescence, remained fluorescence , peak base width and addition volume (final volume)

Table 4: Effect of flow rate on the total, quenching and remained of fluorescence

Speed pump	Flow rate (mL/min)	Total quenched fluorescence expressed as an average peak heights(n=3) $\bar{y}_i$ in mV	RSD%	Confidence interval of the average response (95% confidence level) $\bar{y}_{i(mV)} \pm t_{0.05/2, n-1} \ \sigma_{n-1}/\sqrt{n}$	Quenched fluorescence $\bar{y}_{Qi}$ (n=3)mV	Remained fluorescence $\bar{y}_{Ri}$(n=3)mV	Δt_b (Sec)	t(sec)	V_{add} (mL)	Concentration in mMol.L^{-1} at flow cell	DF
5	0.20	550	0.36	550± 4.92	30	3000	400	72	1.407	0.049	40.21
10	0.45	600	0.29	600±4.32	80	2950	108	66	0.884	0.079	25.26
15	0.80	650	0.56	650±9.04	130	2900	66	42	0.954	0.073	27.26
20	0.90	680	0.23	680±3.89	160	2870	42	36	0.704	0.099	20.11
25	1.00	700	0.22	700±3.83	180	2850	36	30	0.674	0.104	19.26
30	1.30	1006	0.16	1006± 3.99	486	2544	30	24	0.724	0.097	20.69
35	1.55	1010	0.06	1010±1.51	490	2540	24	22	0.694	0.101	19.83
40	1.75	1015	0.07	1015± 1.77	495	2535	18	22	0.599	0.117	17.11

Response of continuous fluorescence : 3550mV, Response of blank : 520mV

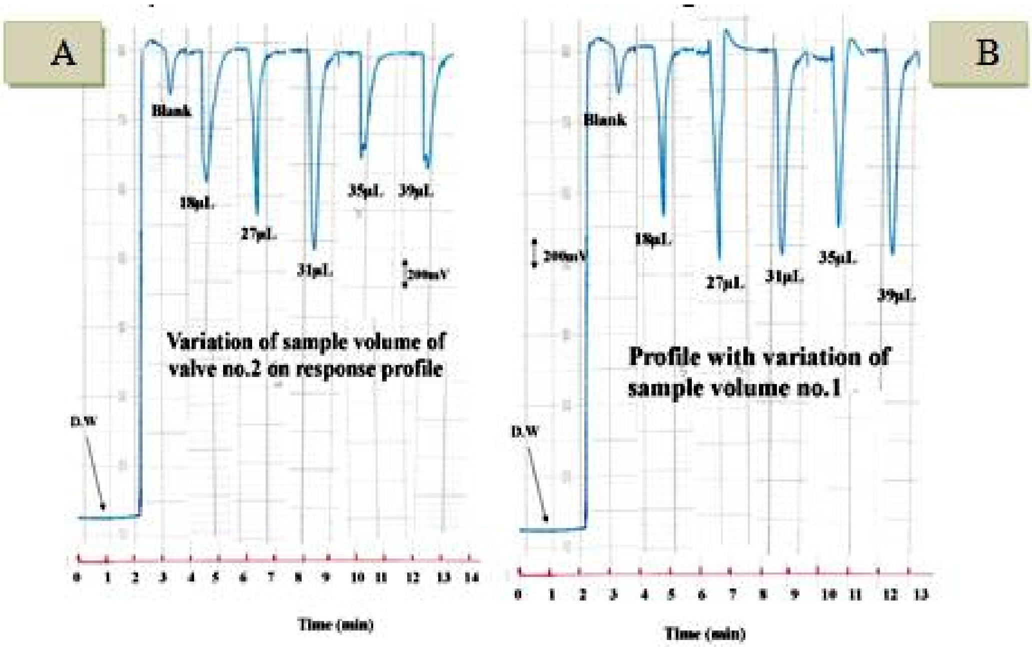
Δt_b (sec) : Time lapse for the fluorescence response within measuring cell or peak base width

t: Departure time for sample segment from injection valve no.$_1$ to the measuring cell

V_{add}: Addition volume(mL) at each flow rate to obtain the final volume , DF: Dilution factor at each flow rate.

3.1.2.2 Sample volume

Using optimum concentration of the reactants: 0.5mMol.L^{-1} fluorescein salt(intensity of the continuous fluorescence) -1mMol.L^{-1} I^- - 0.5mMol.L^{-1} IO_3^--2mMol.L^{-1} HCl at flow rate 1.30mL/min for the optimization of sample volume at valve no.$_2$ (for the complement solution I^--IO_3^-).A variable volume (18-39µL) were injected using sequential open valve mode. The data obtained were plotted as shown in fig.6 which reveals that the optimum sample volume is 31µL that give's a regular and highest clear response . More than 31µL there were a decrease and a distortion of the response profile. Using the optimum parameters achieved previously the injected volume of sample segment (oxonium ion) was varied in the range 18-39µL by the changing the length of the sample loop of injection valve no.$_1$. All results tabulated in table 5. It was noticed that any increase in the sample volume at valve no.$_1$ led to increase in peak height; when using larger volume i.e.more than 27µL it gave a decrease in response profile height (as form quenching (negative response)). Therefore 27µL was found to the best sample volume that gave a sharpregular response(fig.6,B,D).



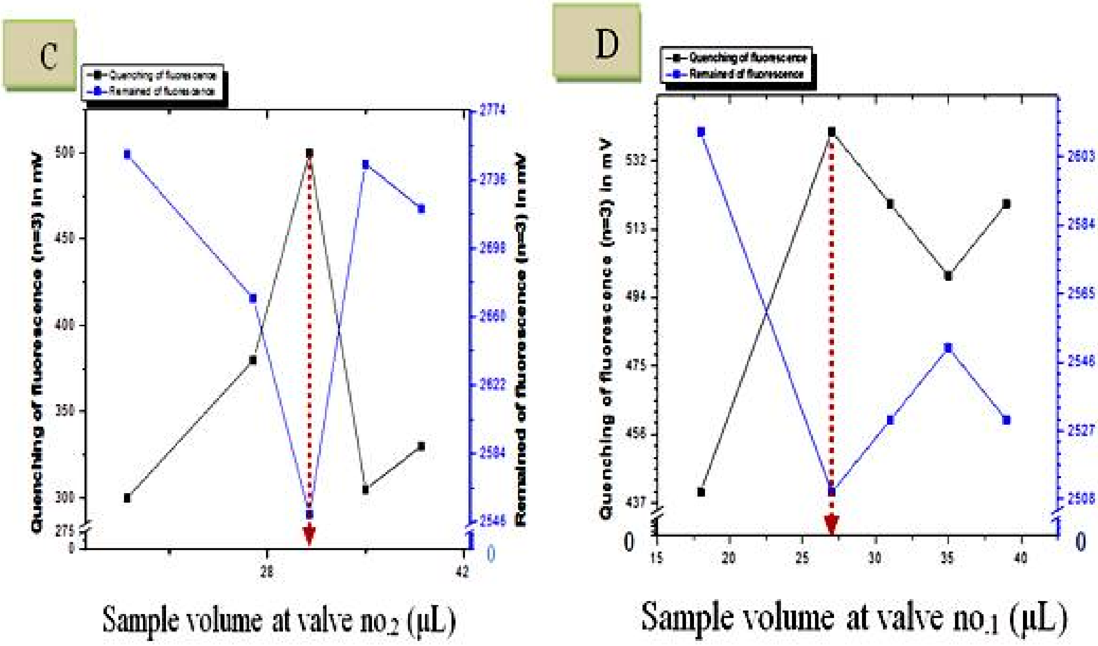


Fig. 6: Effect of sample volume at valve no.$_1$ and sample volume at valve no.$_2$ on : A,B- Response profile. C,D- Quenching of fluorescence response and remained of fluorescence

Table 5: Effect of the variation of injected sample volume (valve no.$_1$ and valve no.$_2$) on the response of the total , quenching and remained fluorescence system

Sample volume (µL)	Total quenched fluorescenceexpressed as an average peak heights (n=3) $\bar{y}_i$ in mV	Quenched fluorescence $\bar{y}_{Qi}$(n=3)mV	Confidence interval of the average response (95% confidence level) $\bar{y}_{i(mV)} \pm t_{0.05/2,\, n-1}\ \sigma_{n-1}/\sqrt{n}$	RSD%	Remained fluorescence $\bar{y}_{Ri}$(n=3)mV
Sample volume no.$_2$					
18	800	300	800±2.78	0.14	2750
27	880	380	880±2.40	0.11	2670
31	1000	500	1000±0.75	0.03	2550
35	805	305	805±0.99	0.05	2745
39	830	330	830±0.62	0.03	2720
Sample volume no.$_1$					
18	940	440	940±1.63	0.07	2610
27	1040	540	1040±5.17	0.20	2510
31	1020	520	1020±4.81	0.19	2530
35	1000	500	1000±2.48	0.10	2550
39	1020	520	1020±4.81	0.19	2530

Response of continuous fluorescence : 3550mV,Response of blank : 500mV

3.1.2.3 Purge time

Using the optimum parameters achieved in previous sections, allowed permissible time for the sample to be injected sequentially between valve no.$_1$ and valve no.$_2$ was studied. The obtained results were tabulated in table 6 which shows the average of total quenching successive measurement expressed in mV, relative standard deviation , the confidence interval of average response at 95% confidence level , quenching of the fluorescence and remained of the fluorescence. It was found that the opening of valve no.$_1$ followed by the second one in a successive manner affect on the profile response and causes a distortion(fig.7.A). Therefore it was found the researcher aim to direct our attention to the period of time between the opening of both valves in a sequential manner. It was found that increasing the lag time period between the two valves up to 32seconds gave a highest peak response beyond 32second. It was noticed a decrease in response profile with a peak distortion. Therefore on this above mentioned behaviour ; 32 seconds, was the choice of time lag between the two valves(fig.7.B). Thus will improve sensitivity and work can be directed to a low detection limit and might improve linearity.

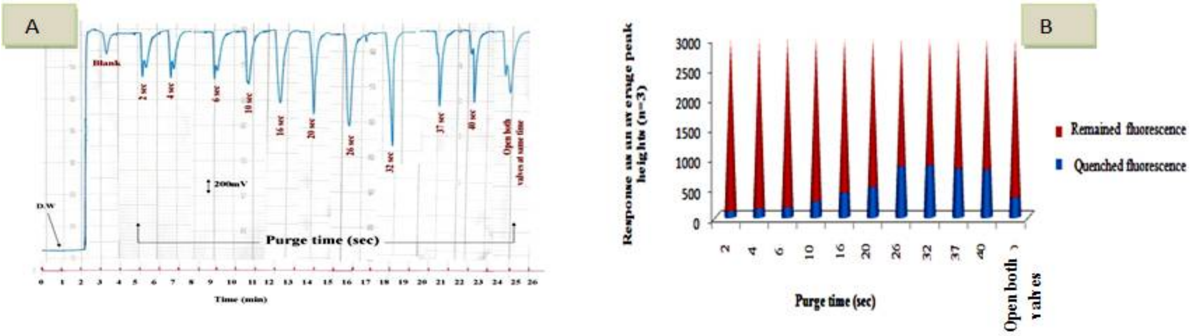


Fig. 7: Effect purge time on: A-Response profile, B- Measurement of quenching fluorescence and remained of fluorescence

Table 6: Variation of purge time on the fluorescence response using I^--IO_3^--H_3O^+ system

Purge time (sec)	Total quenched fluorescence expressed as an average peak heights (n=3) $\bar{y}_i$ in mV	Quenched fluorescence $\bar{y}_{Qi}$ (n=3)mV	RSD%	Confidence interval of the average response (95% confidence level) $\bar{y}_{i(mV)} \pm t_{0.05/2,\ n-1}\ \sigma_{n-1}/\sqrt{n}$	Remained fluorescence $\bar{y}_{Ri}$(n=3)mV
2	620	120	0.04	620±0.62	2880
4	660	160	0.2	660±3.28	2840
6	680	180	0.09	680±1.52	2820
10	780	280	0.11	780±2.13	2720
16	920	420	0.33	920±7.54	2580
20	1020	520	0.09	1020±2.28	2480
26	1360	860	0.08	1360±2.70	2140
32	1380	880	0.05	1380±1.71	2120
37	1320	820	0.15	1320±4.92	2180
40	1300	800	0.16	1300±5.17	2200
Open both valves at the same time	840	340	0.13	840±2.71	2660

Lag time or purge time : sequential manner i.e; opening valve no.$_1$ followed by the second valve.Response of continuous fluorescence : 3500mV, Response of blank : 500Mv.

3.1.2.4 Reaction coil

Using optimum concentration for quenching system (1mMol.L^{-1} I^--0.5mMol.L^{-1}IO_3^- -2 mMol.L^{-1}HCl) with fluorescein salt solution 0.5mMol.L^{-1} and using physical parameters achieved in previous studies with variable coil length 0-100cm. Fig.8 and table 7 shows and tabulated all the obtained results. It was noticed a decrease in the response profile obtained when using reaction coil this might be attributed to the dilution and dispersion effect.

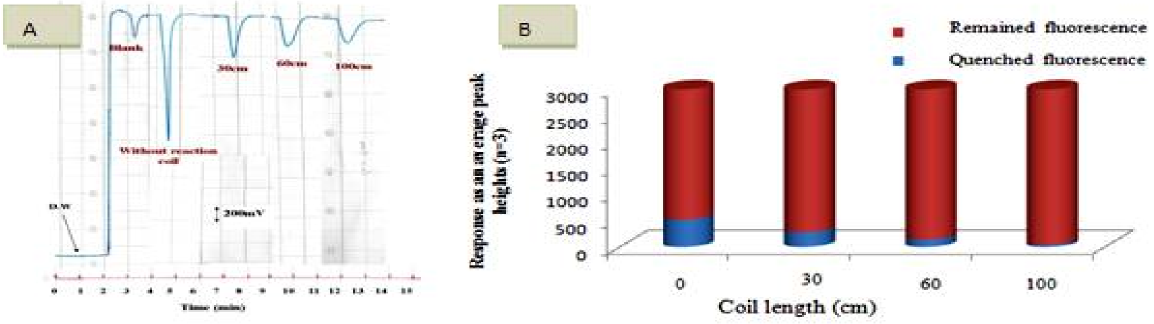


Fig. 8: Effect of reaction coil on : A-Response profile B-quenching of fluorescence response and remained fluorescence

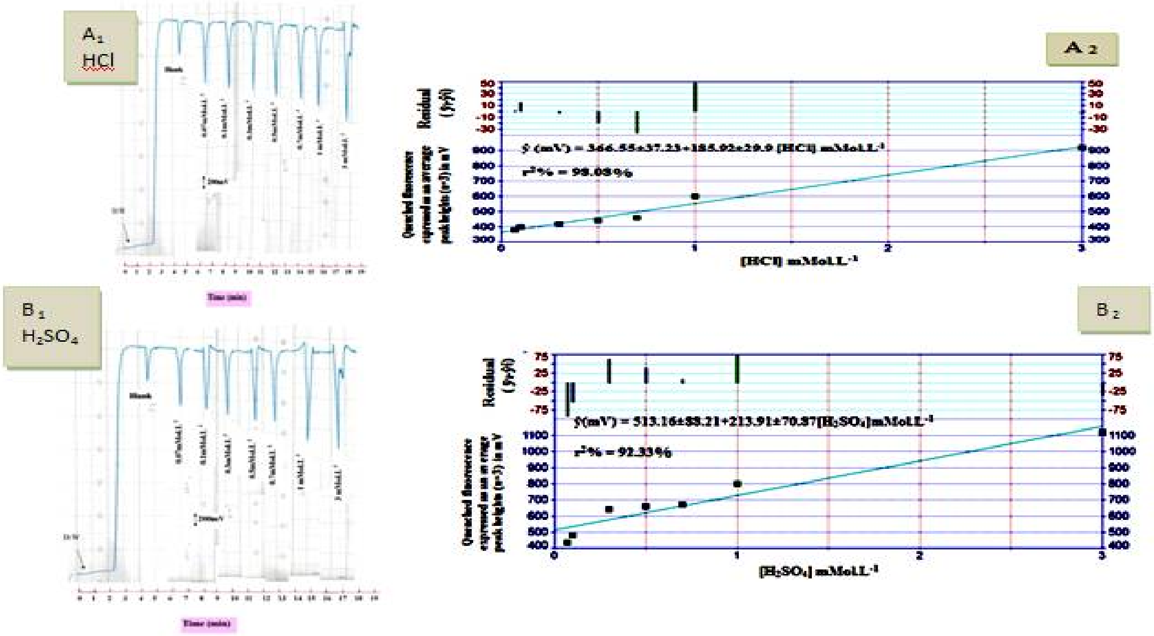
Table 7: Variation of reaction coil with total, quenching and remained fluorescence

Coil length (cm)	Coil volume(mL) $r^2\pi h$ r=0.5mm	Total quenched fluorescence expressed as an average peak heights (n=3) $\bar{y}_i$ in mV	Quenched fluorescence $\bar{y}_{Qi}$ (n=3)mV	RSD%	Confidence interval of the average response (95% confidence level) $\bar{y}_{i(mV)} \pm t_{0.05/2,\, n-1}\ \sigma_{n-1}/\sqrt{n}$	Remained fluorescence $\bar{y}_{Ri}$(n=3)mV
0	0	1340	840	0.11	1340±3.66	2160
30	0.235	780	280	0.22	780±4.26	2720
60	0.471	640	140	0.11	640±1.75	2860
100	0.785	540	40	0.28	540±3.76	2960

Response of continuous fluorescence : 3500mV,Response of blank : 500mV

3.2 Calibration curve

A series of oxonium ion concentration (HCl,H_2SO_4,$HClO_4$ and tartaric acid) having the range of (0.0005-7)mMol.L^{-1} were papered using all achieved parameters in previous sections. A responses profile and a straight lines for all acids were obtained as shown in fig.9. Table 8 sum up all the results obtained using linear regression analysis.



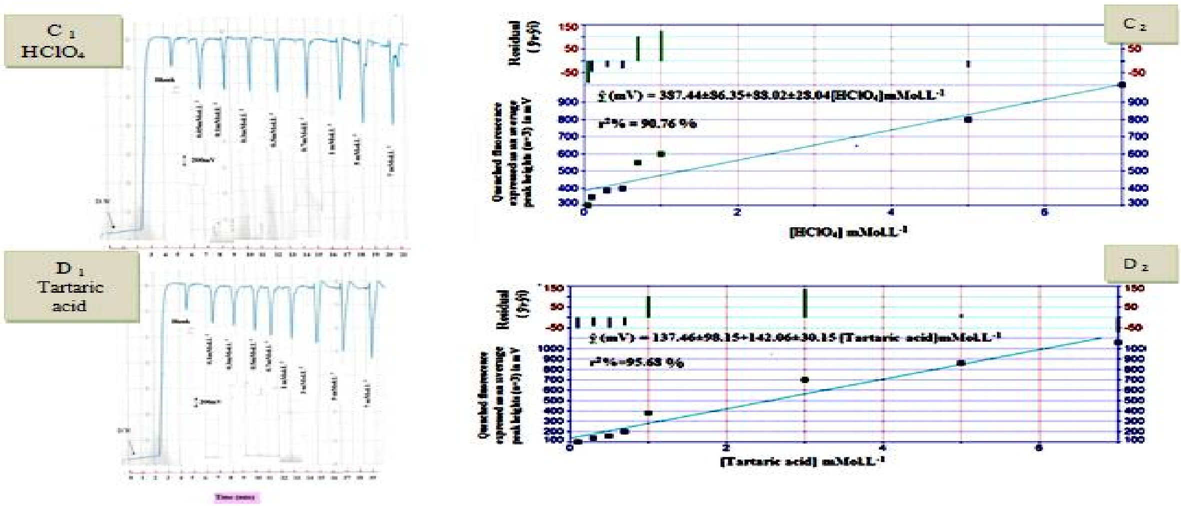


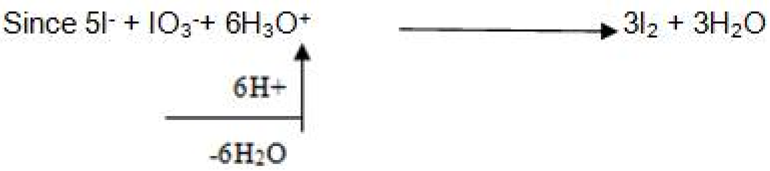
Fig. 9: Calibration graph for the variation of oxonium ion concentration (HCl, H_2SO_4 , $HClO_4$ and tartaric acid) on : A_1,B_1,C_1 andD_1-Response profile .A_2,B_2,C_2 andD_2-Quenching of fluorescence response expressed by linear equationusing Laser Diode Fluorimeter. Residual = ($\bar{y}$i-ŷi) in mV, $\bar{y}$i = practical value,ŷi =estimated value

Table 8: Summary of calibration curve results for the determination of oxonium ion from (HCl, H_2SO_4 , $HClO_4$ and tartaric acid) using quenched fluorescence

Type of acid	Measured $[H_3O^+]$ mMol.L^{-1}	Linear dynamic range mMol.L^{-1}	Type of measurement	ŷ(mV)=(a± S_at)+ (b±S_bt) $[H_3O^+]$ at confidence level 95%, n-2	r r^2 r^2%	t_{tab} at 95% confidence level, n-2	$t_{cal}=\frac{\lvert r\rvert\sqrt{n-2}}{\sqrt{1-r^2}}$
HCl Ka=10^6	0.0005-7	n=7 0.07-3	Total quenching of fluorescence	866.55±37.23+185.92±29.90 [HCl]mMol.L^{-1}	0.9904 0.9808 98.08	2.571<< 15.98	
			Quenching of fluorescence	366.55±37.23+185.92±29.90 [HCl] mMol.L^{-1}			
			Remained of fluorescence	2633.45±37.23-185.92±29.90 [HCl] mMol.L^{-1}			
H_2SO_4 Ka=10^3	0.0005-7	n=7 0.07-3	Total quenching of fluorescence	1013.16±88.21+213.91±70.87 $[H_2SO_4]$ mMol.L^{-1}	0.9609 0.9233 92.33	2.571<< 7.76	
			Quenching of fluorescence	513.16±88.21+213.91±70.87 $[H_2SO_4]$ mMol.L^{-1}			
			Remained of fluorescence	2486.84±88.21-213.91±70.87 $[H_2SO_4]$ mMol.L^{-1}			
$HClO_4$ Ka=10^3	0.0005-7	n=8 0.05-7	Total quenching of fluorescence	887.44±86.35+88.02±28.04 $[HClO_4]$ mMol.L^{-1}	0.9527 0.9076 90.76	2.447<< 7.68	
			Quenching of fluorescence	387.44±86.35+88.02±28.04 $[HClO_4]$ mMol.L^{-1}			
			Remained of fluorescence	2612.56±86.35-88.02±28.04 $[HClO_4]$ mMol.L^{-1}			
Tartaric acid Ka_1= 1.29x10^{-3} Ka_2= 3.98x10^{-5}	0.0005-7	n=8 0.1-7	Total quenching of fluorescence	637.46±98.15+142.06±30.15 [tartaric acid] mMol.L^{-1}	0.9782 0.9568 95.68	2.447<< 11.53	
			Quenching of fluorescence	137.46±98.15+142.06±30.15 [tartaric acid] mMol.L^{-1}			
			Remained of fluorescence	2862.54±98.15-142.06±30.15 [tartaric acid] mMol.L^{-1}			

ŷ: estimated response (mV) for (n=3) expressed as an average peak heights of linearequation of the form ŷ= a+bx, $[H_3O^+]$: acid concentration (mMol.L^{-1}), r :correlation coefficient, r^2:coefficient of determination, r^2%: linearity percentage

-In order to explain the behaviour of the profile of the scatter plot from which a calibration graph can be depicted the following most probable explanation can be used.

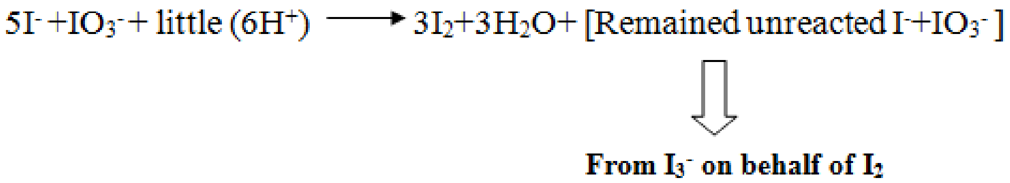


$$\text{Since } 5I^- + IO_3^- + 6H_3O^+ \longrightarrow 3I_2 + 3H_2O \qquad 6H^+,\ -6H_2O$$

Iodine is solid but in excess of I^- it form a soluble product of triiodide ion I_3^-; this in turn might lead to a constant of response height, due to I_3^- does not affect on the quenching of fluorescence intensity compared with I_2.

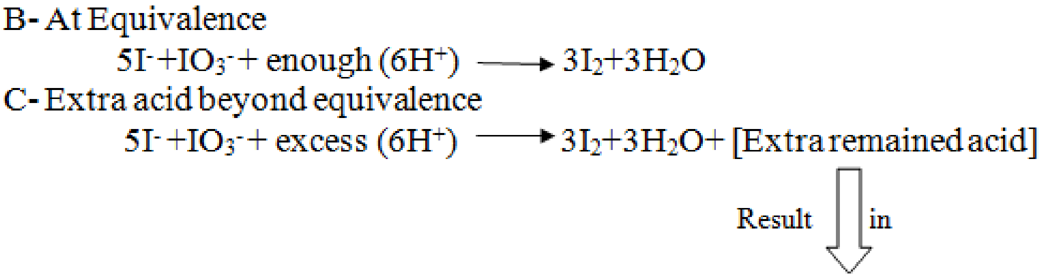
Since the I^-& IO_3^- are available at relatively high level at low acid concentration which might reach a state of equivalence at a certain level of acid concentration. While at high level of acid concentration extra acid will be remained due to unavailability of I^- / IO_3^- necessary to form iodine. All this can be explained by the following equation

A – At low acid concentration



$$5I^- + IO_3^- + \text{little } (6H^+) \longrightarrow 3I_2 + 3H_2O + [\text{Remained unreacted } I^- + IO_3^-]$$

From I_3^- on behalf of I_2



B- At Equivalence

$$5I^- + IO_3^- + \text{enough } (6H^+) \longrightarrow 3I_2 + 3H_2O$$

C- Extra acid beyond equivalence

$$5I^- + IO_3^- + \text{excess } (6H^+) \longrightarrow 3I_2 + 3H_2O + [\text{Extra remained acid}]$$

Result in

It will affect the solubility of uranine salt by formation of fluorescein free acid which is not soluble in acid medium. Thus form a tiny most probably a microscale particulate; that will distord the signal profile

- The above mentioned scheme explains the distortion of the quenched distorted energy transducer response versus time profile at high acid concentration.

From the results were tabulated in table 8 which were used in order to distinguish between the four different acids and does the four different acids give the same H_3O^+ output i.e. acid variation and they contribution the same effect.

Therefore If it is assumed that there is no significant difference between the mean of the measurement of acid type (HCl, H_2SO_4, $HClO_4$ and tartaric acid) and measurement (Null hypothesis)

H_o : $\mu_{\text{Hydrochloric acid}} = \mu_{\text{Sulphuric acid}} = \mu_{\text{Perchloric acid}} = \mu_{\text{Tartaric acid}}$ Against

The alternative hypothesis H_1: : $\mu_{\text{Hydrochloric acid}} \neq \mu_{\text{Sulphuric acid}} \neq \mu_{\text{Perchloric acid}} \neq \mu_{\text{Tartaric acid}}$

Carrying out ANOVA test[17,18] (table 9) reveal a significant value of 0.00 which is less than the criteria value alpha (0.05). Thus indicate accepting the alternative hypothesis on behalf of the Null hypothesis i.e.; the mean of different measurements is significantly different for each acid used.

Table 9: ANOVA results of laser diode fluorimeter for the determination of H_3O^+ from different acids

Type of test (source of variation)	Sum of square SSq	Degree of freedom (df)	Mean square Msq	F_{cal}	F_{tab}	Sig
Concentration	738211.875	$\upsilon_1 = 5$	147642.375	41.996	2.91	0.00<0.05 (sig)
Acid type	609593.458	$\upsilon_1 = 3$	203197.819	57.757	3.29	0.00<0.05 (sig)
Error	52772.292	$\upsilon_2 = 15$	3518.153			
Total	1400577.625	23				

$F_{tab} = F_{\alpha}, \upsilon_1, \upsilon_2 = F_{0.05}, \upsilon_1, \upsilon_2$

3.2.1 Detection limit

Limit of detection for the determination of oxonium ion (HCl, H_2SO_4 , $HClO_4$ and tartaric acid) using laser diode fluorimeter was studied. The results is tabulated in table 10 using 27µL as a sample volume.

Table 10: Detection limit of all acids using laser diode fluorimeter

Type of acids	Minimum* concentration ($mMol.L^{-1}$)	Practical based on the gradual dilution for the minimum concentration (D.L)	Theoretical based on the value of slope $X=3S_B/slope$
HCl	0.0001	0.09ng/sample	0.13 ng/sample
H_2SO_4	0.0001	0.26ng/sample	0.11ng/sample
$HClO_4$	0.0001	0.27ng/sample	0.28ng/sample
Tartaric acid	0.0001	0.41ng/sample	0.17ng/sample

X: value of L.O.D based on slope, S_B: Standard deviation of blank

*D.L : Minimum concentration from gradual dilution of the minimum concentration in calibration graph

3.2.2 Repeatability

The repeatability of measurements and the efficiency of adopted method (laser diode fluorimeter) were studiedat concentration of oxonium ion : 0.9 $mMol.L^{-1}$ (HCl) , 0.3 $mMol.L^{-1}$ (H_2SO_4) , 1 $mMol.L^{-1}$ ($HClO_4$) and 2$mMol.L^{-1}$ (tartaric acid) using the optimum parameters achieved in previously study. Fig.10 and table 11 shows and tabulate the results obtained.

Table 11: Repeatability of HCl, H_2SO_4, $HClO_4$ and tartaric acid results obtained for the I^-- IO_3^--H_3O^+ system

Type of acid	Concentration $mMol.L^{-1}$	Average of total quenched fluorescence expressed as an average peak heights $\bar{y}_i$ in mV	Quenched fluorescence $\bar{y}_{Qi}(n=3)$mV	RSD%	Confidence interval of the average response (95% confidence level) $\bar{y}_{i(mV)} \pm t_{0.05/2, n-1} \ \sigma_{n-1}/\sqrt{n}$
HCl	0.9	1040	540	0.05	1040±0.43
H_2SO_4	0.3	1140	640	0.13	1140±1.24
$HClO_4$	1	1100	600	0.07	1100±0.64
Tartaric acid	2	1080	580	0.09	1080±0.81

Response of continuous fluorescence=3500mV, Response of blank : 500mV,$t_{0.05/2, 7=2.365}$

Number of injection = 8

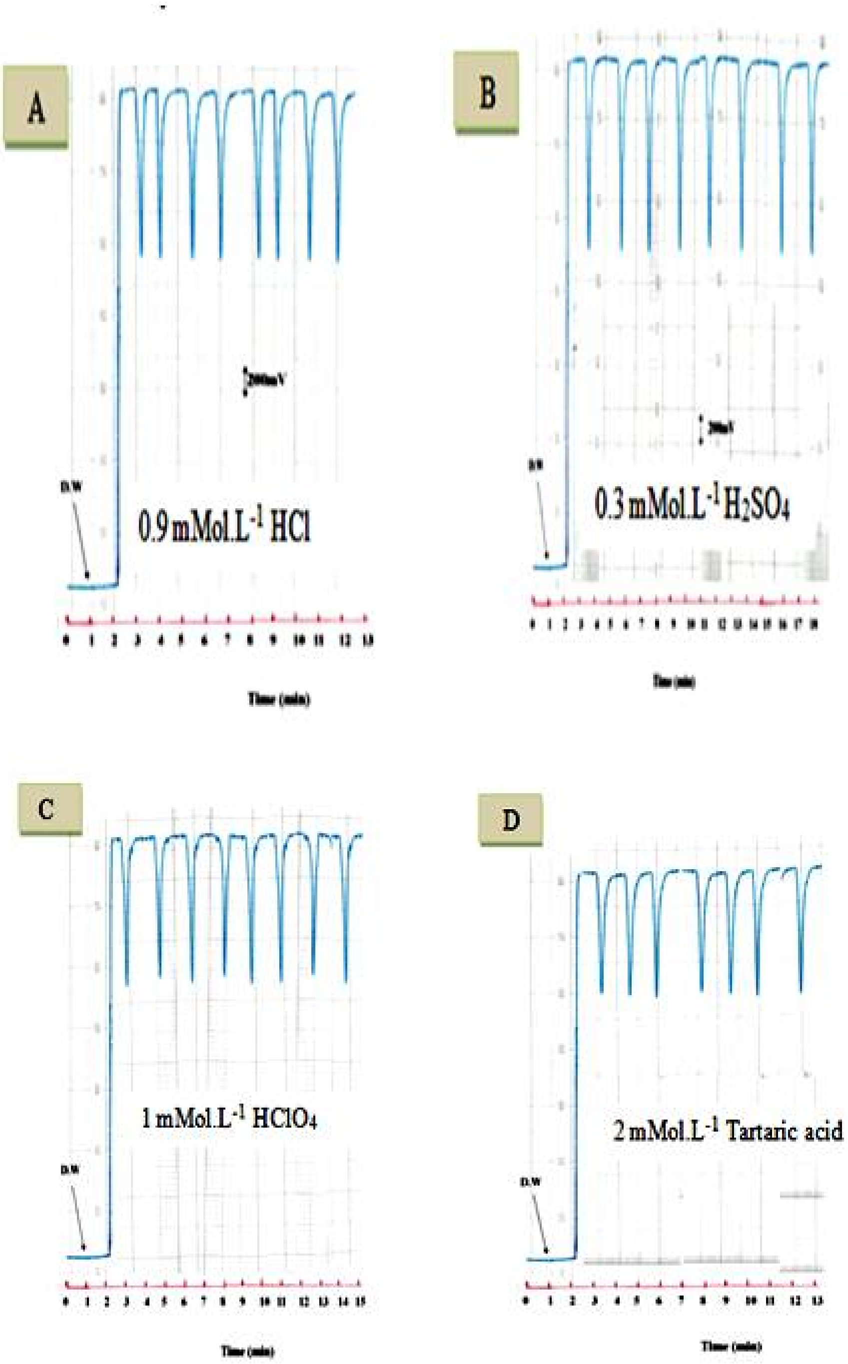


Fig. 10: Eight successive repeatable measurement using quenching fluorescence response of A: HCl , B:H_2SO_4, C:$HClO_4$ and D: tartaric acid

3.3 Evaluation of the use of laser diode fluorimeter in the determination of two different acids in commercial samples as an application

Two different samples of acids (H_2SO_4, 98%, LobaChemie-India & tartaric acid , Thomas Baker-India) were used to analyze and determined of oxonium ion. Continuous flow injection analysis using laser diode fluorimeter achieved in this work and was compared by classical method (pH-meter) via the measurement of pH, calibration curve was obtained for pure acids (HCl, H_2SO_4, $HClO_4$& tartaric acid) with range of concentration of 0.005-7mMol.L^{-1}. The results for linear regression equation summed up in table 12. The preparation of standard addition calibration curve for two methods and each sample were prepared by transferring 0.05mL(50mMol.L^{-1}) of the five volumetric flask (25mL), followed by different aliquots of 0.0, 0.1, 0.2, 0.3 & 0.4 mL from 50mMol.L^{-1} standard solution of H_2SO_4& tartaric acid in order to have the concentration range 0.0-0.8mMol.L^{-1}. Table 13.A shows the summary of standard addition method results from two samples using two methods. A straight- line graph from 0.0-0.8 mMol.L^{-1} were obtained (fig.11).

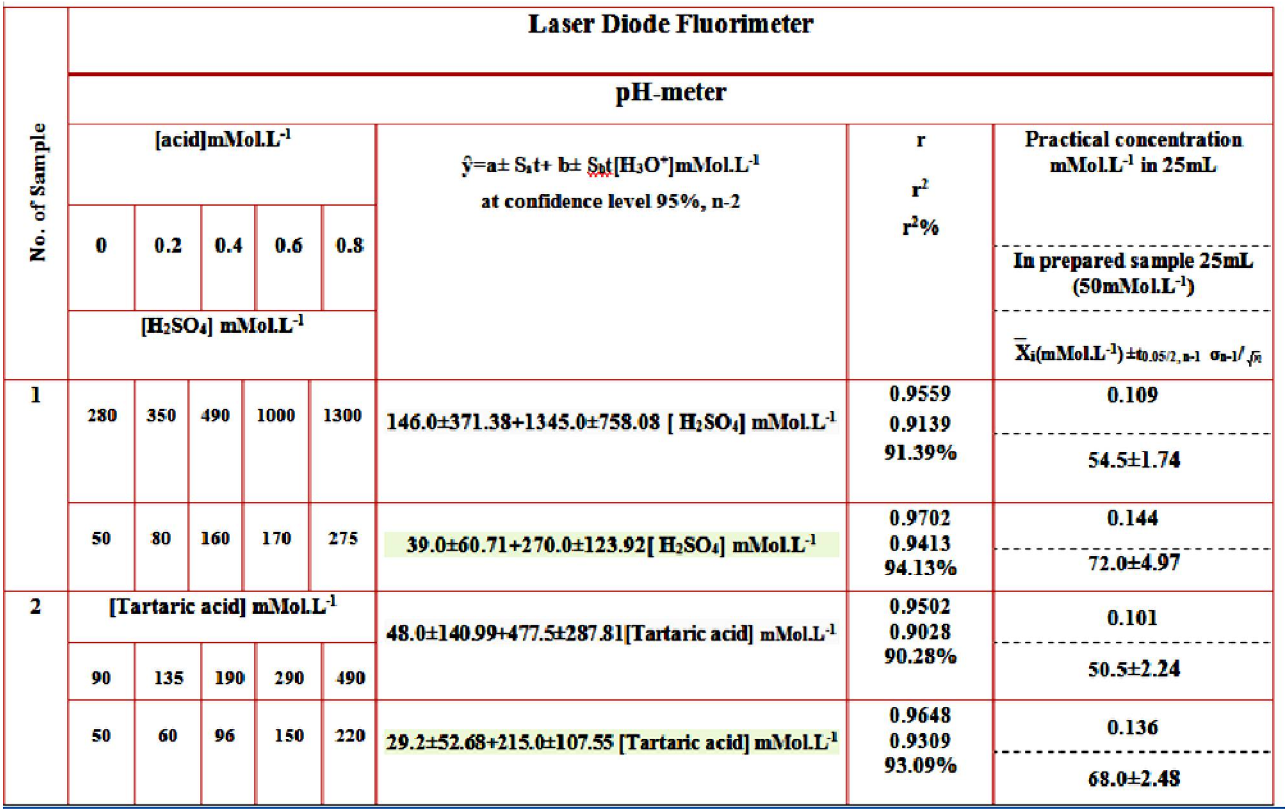
A comparison was made between the FIA-laser diode fluorimeter and pH-method with value (50mMol.L^{-1}) . The obtained values suggest that there is no significant difference between 50mMol.L^{-1} and calculated t values for each individual t-test of both method (as the calculated t-value is less than critical tabulated t-value) . Therefore any one of them can be used for determination of H_3O^+ from H_2SO_4 or tartaric acid. The obtained results as shown in table 13.B at 95% confidence interval.

Table 12: Summary of calibration graph results for the determination of oxonium ion using pH-meter

Type of acid	Measured $[H_3O^+]$ mMol.L^{-1}	$\hat{y}$(mV)=(a± S_at)+(b±S_bt)$[H_3O^+]$mMol.L^{-1} at confidence level 95%, n-2	r r^2 r^2%	t_{tab} at 95% confidence level, n-2	$t_{cal}=\frac{\lvert r\rvert\sqrt{n-2}}{\sqrt{1-r^2}}$
HCl	n=8 0.005-7	97.33±32.66+37.28±12.00[HCl]mMol.L^{-1}	0.9518 0.9059 90.59%	2.447<<7.60	
H_2SO_4	n=8 0.005-7	96.79±33.82+42.39±12.42[H_2SO_4] mMol.L^{-1}	0.9595 0.9207 92.07%	2.447<<8.35	
$HClO_4$	n=7 0.005-7	108.58±16.44+63.78±13.21[$HClO_4$] mMol.L^{-1}	0.9842 0.9686 96.86%	2.571<<12.41	
Tartaric acid	n=8 0.005-3	74.38±24.10+76.55±20.79[Tartaric acid] mMol.L^{-1}	0.9649 0.9312 93.12%	2.447<<9	

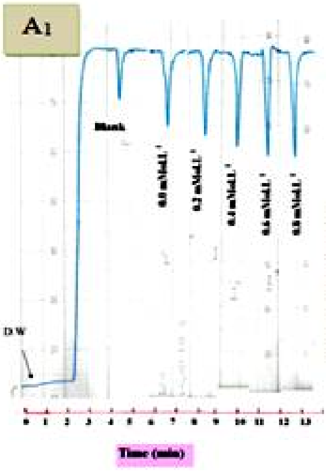
$\hat{y}$: estimated response (mV) for n=3 expressed as an average peak heights of linear equation of the form $\hat{y}$= a+bx, $[H_3O^+]$: acid concentration (mMol.L^{-1}), r :correlation coefficient, r^2:coefficient of determination,r^2%:linearity percentage

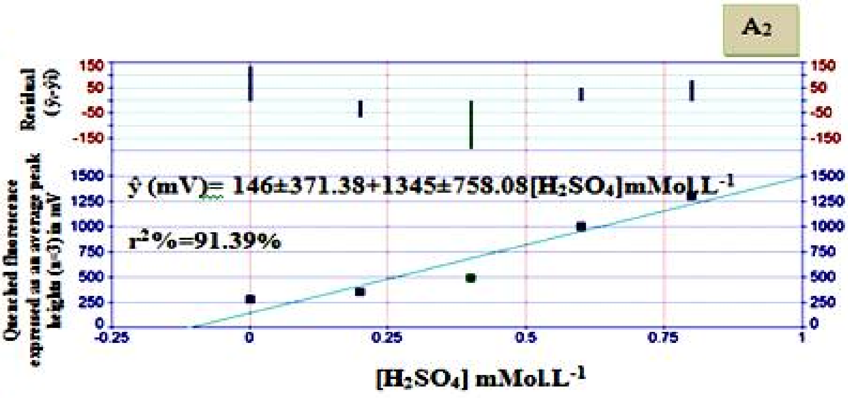
Table 13A: Summary of results by standard additions method for the determination of oxonium ion by quenching of fluorescence system using laser diode fluorimeter method and pH-meter method

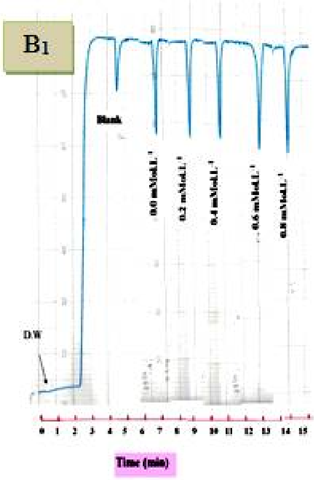


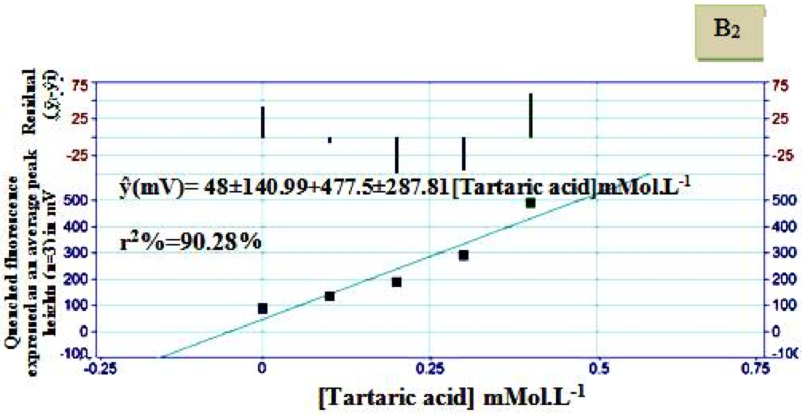
No. of Sample	Laser Diode Fluorimeter							
	pH-meter							
	[acid]mMol.L^{-1}					$\hat{y}$=a± S_at+ b± S_bt$[H_3O^+]$mMol.L^{-1} at confidence level 95%, n-2	r r^2 r^2%	Practical concentration mMol.L^{-1} in 25mL In prepared sample 25mL (50mMol.L^{-1}) $\bar{X}_i$(mMol.L^{-1})±$t_{0.05/2,n-1}$ $\sigma_{n-1}/\sqrt{n}$
	0	0.2	0.4	0.6	0.8			
	$[H_2SO_4]$ mMol.L^{-1}							
1	280	350	490	1000	1300	146.0±371.38+1345.0±758.08 [H_2SO_4] mMol.L^{-1}	0.9559 0.9139 91.39%	0.109 54.5±1.74
	50	80	160	170	275	39.0±60.71+270.0±123.92[H_2SO_4] mMol.L^{-1}	0.9702 0.9413 94.13%	0.144 72.0±4.97
2	[Tartaric acid] mMol.L^{-1}					48.0±140.99+477.5±287.81[Tartaric acid] mMol.L^{-1}	0.9502 0.9028 90.28%	0.101 50.5±2.24
	90	135	190	290	490			
	50	60	96	150	220	29.2±52.68+215.0±107.55 [Tartaric acid] mMol.L^{-1}	0.9648 0.9309 93.09%	0.136 68.0±2.48

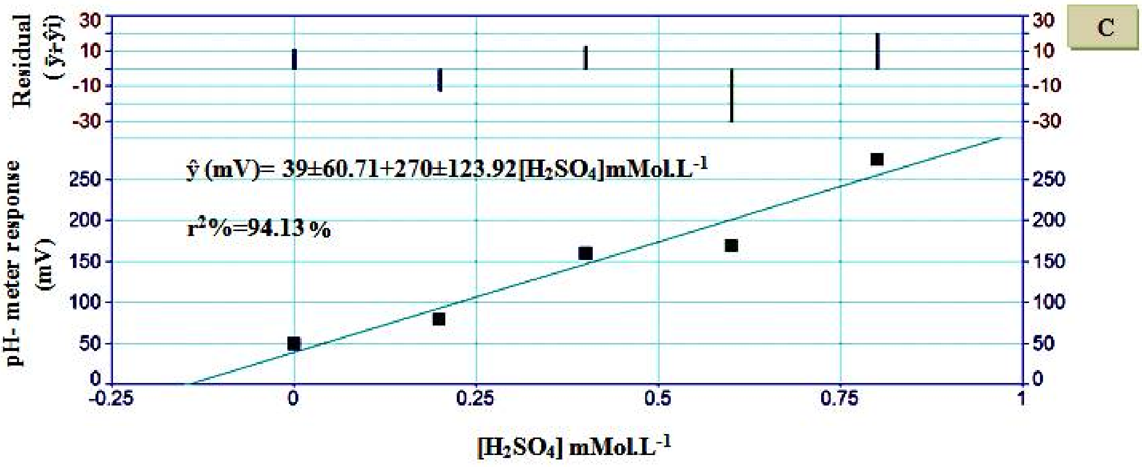
$\hat{y}$: Estimated response value (mV for laser diode fluorimeterand pH-meter method) for n=3, $[H_3O^+]$:Acid concentration (mMol.L^{-1}), r :correlation coefficient, r^2:coefficient of determination& r^2%: linearity percentage, $t_{0.05/2,\ 2=4.303}$


A1
Blank
0.0 mMol.L-1
0.2 mMol.L-1
0.4 mMol.L-1
0.6 mMol.L-1
0.8 mMol.L-1
D.W
0 1 2 3 4 5 6 7 8 9 10 11 12 13
Time (min)


A2
Residual (ŷi-yi)
150
50
-50
-150
Quenched fluorescence expressed as an average peak heights (n=3) in mV
1500
1250
1000
750
500
250
0
ŷ (mV)= 146±371.38+1345±758.08[H2SO4]mMol.L-1
r2%=91.39%
-0.25
0
0.25
0.5
0.75
1
[H2SO4] mMol.L-1


B1
Blank
0.0 mMol.L-1
0.2 mMol.L-1
0.4 mMol.L-1
0.6 mMol.L-1
0.8 mMol.L-1
D.W
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
Time (min)


B2
Residual (ŷi-yi)
75
25
-25
Quenched fluorescence expressed as an average peak heights (n=3) in mV
500
400
300
200
100
0
-100
ŷ(mV)= 48±140.99+477.5±287.81[Tartaric acid]mMol.L-1
r2%=90.28%
-0.25
0
0.25
0.5
0.75
[Tartaric acid] mMol.L-1


C
Residual (ŷi-yi)
30
10
-10
-30
pH- meter response (mV)
250
200
150
100
50
0
ŷ (mV)= 39±60.71+270±123.92[H2SO4]mMol.L-1
r2%=94.13 %
-0.25
0
0.25
0.5
0.75
1
[H2SO4] mMol.L-1

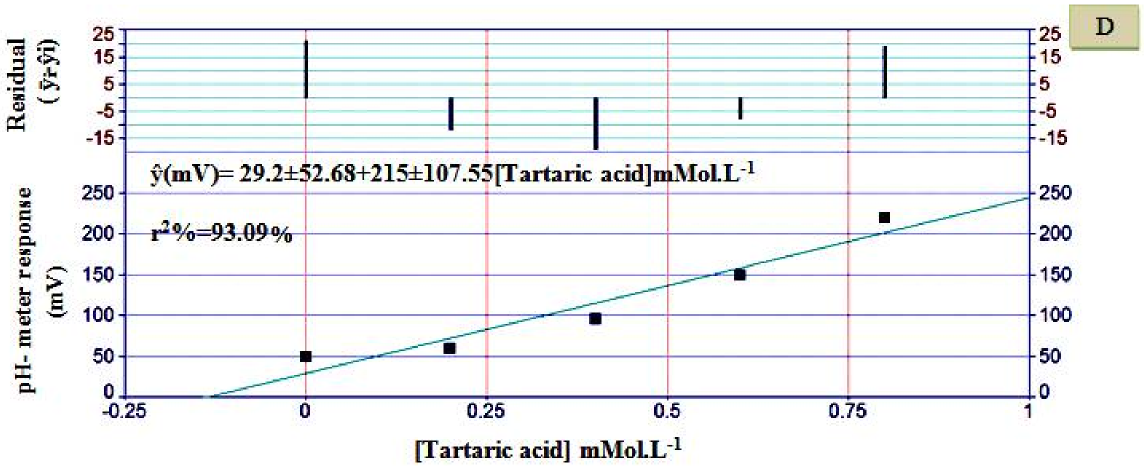


Fig. 11: Standard addition of response profile and calibration graph using laser diode fluorimeter for : A_1,A_2- H_2SO_4,B_1,B_2- Tartaric acid and calibration graph usingpH-meter for: C- H_2SO_4 D- Tartaric acid Residual = (ȳi-ŷi) in mV, ȳi = practicalvalue, ŷi =estimated value

Table 13B: Paired t-test for comparison between standard value and practically value for two differentacids using two methods

$[H_3O^+]$ (mMol.L^{-1})	Laser diode fluorimeter			
Standard value 50	pH-meter			
	H_2SO_4		Tartaric acid	
Practically value	54.5		50.5	
	72.0		68.0	
	$\bar{x}_{1d}=13.25$ $\sigma_{n-1}=12.37$ n=2 df=1		$\bar{x}_{2d}=9.25$ $\sigma_{n-1}=12.37$ n=2 df=1	
	$t_{cal}=\text{б}_{d}\sqrt{n}/\sigma_{n-1}$	t_{tab}	$t_{cal}=\text{б}_{d}\sqrt{n}/\sigma_{n-1}$	t_{tab}
	1.515<< 12.706		1.057 << 12.706	

$\bar{x}_{1d}$: average of difference between practically value for sulphuric acid with standard value , $\bar{x}_{2d}$: average of difference between practically value for tartaric acid with standard value , $t_{tab=}t_{0.05/2,\ n-1}$ =12.706 (for n=2) for paired t-test,t_{cal}= value of t-calculated for paired test, n: no.of methods, df: degree of freedom(n-1) , σ_{n-1} : standard deviation

CONCLUSIONS

The proposed method characterized by simplicity, speed and accuracy for determination of oxonium ion by continuous flow injection analysis coupled with laser diode fluorimeter. The RSD% less than 1% was observed for all samples, indicating a satisfactory precision of the proposed method. The standard addition method was used to avoid matrix effects. A final conclusion can drawn that the newly adopted analysis method for the determination of oxonium ion can be regarded as an alternation assessment at confidence of 95% (α=0.05).

ACKNOWLEDGEMENT

I would like to express my deepest gratitude to Prof. Dr. IssamM.A.Shakir Al-Hashimi for his appreciable advice, important comments, support and encouragement.

REFERENCES

1. Michael M. Principles of chemistry ,2000,1nd Edition, w.w.Norton and company , Inc, New York, 84-86.
2. Martin S. Chemistry,2006, 4nd Edition, McGraw-Hill companies, Inc, New York, 768-772.
3. Ebbing D. General chemistry.2005, 8nd Edition, Boston , Houghton Mifflin.
4. Caenen P and Daerden M. Mechanism of single and multiple step pickling of stanless steal in acid. Journal of corrosion science. 2005;47(5):1307-1324.
5. Amdu M. Health effect of air pollutants: sulphuric acid, the old and new. Journal of health and perspect.1989;81(1):109-113.

6. Sahin F, Volkan M and Ataman OY. Effect of nitric acid for equal stabilization and sensitivity of different selenium species in electrothermal atomic absorption spectrometry. Analytical Chemica Acta. 2005;547(1):126-131.
7. Bucker S and Acker J. Spectrometric analysis of process eating solutions of the photovoltaic industry-determination of HNO_3, HF and H_2SiF_6 usingheigh-resolution continuum source absorption spectrometry of diatomic molecules and atoms. Talanta. 2012;94:335-341.
8. Wenke W, Jörg A and Iris G. Determination of total fluoride in HF/HNO_3/H_2SiF_6eatch solutions by new potentiometric titration methods. Talanta. 2007;71(5):1901-5.
9. Issam MA, Al-Hashimi and Hussein FA and Al-Razack. New mode for the on-line determination of oxonium ion in different strong acids using CFIA via the use of homemade linear array Ayah 5SX4-ST-5D solar CFI analyser.Iraqi journal of science. 2015;56(1):282-302.
10. Issam MA, Al-Hashimi and Ahmad AM. Novel method for determination of hydrochloric acid via ion exchange and precipitation reaction indirectly by using linear array 5SX1-T-1D continuous flow injection analyser.International journal of research in pharmacy and chemistry. 2014;4(4):763-776.
11. Issam MA, Al-Hashimi and Hussein FA and Al-Razack. A novel semiautomated on-line determination of oxonium ion via precipitation reaction using a new mode of attenuated measurement of incident light(0-180^0) by Ayah 5SX4-ST-5D solar CFI analyser. Iraqi journal of science .2014;55(4):1736-1750.
12. Issam MA, Al-Hashimi, Dakhil NT and FalahH H. Hydronium ion determination via on-line semiautomated merging zone (homemade unite).National journal of chemistry. 2002;6:231-245.
13. Issam MA, Al-Hashimi and Azad TF. Determination of oxonium ion in strongly ionisable inorganic acids and determination of substituted acetic acids using flow injection and chemiluminescence detectection. Analyst.1990;115:69-72.
14. Issam MA, Al-Hashimi and Ra'ad FH. Anovel method for the determination of oxonium ion in strong acids via flow injection-chemiluminescence. 2009. MSc.Thesis, University of Baghdad.
15. Joseph RL. Principles of fluorescence spectrometry,1983, 3nd Edition, Baltimone, Maryland,USA.
16. Berlman IB. Handbook of fluorescence spectra of aromatic molecules.1971;2ndEdition, Academic press, New York.
17. Miller JM and Miller JC. Stastical and chemometric for analytical chemistry. 2005; 5nd Edition , Person eduction limited.
18. Miller JC and Miller JN. Statistical for analytical chemistry.1988,2nd Edition, Jhonwilley and N.Y.Sons.