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Determination of oxonium ion and application via quenching of continuous fluorescence light (fluorescein salt) using I^- -IO_3^--H_3O^+ system

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Abstract

The research work present a sensitive, accurate and fast developed for the determination of oxonium ion (HCl, H_2SO_4 , $HClO_4$ and tartaric acid). It relies on the formation free iodine molecule from the I^--IO_3^--H_3O^+ reaction which react with fluorescein sodium salt solution causing to quench the fluorescence light (continuous fluorescence) when irradiated by laser source at 405nm. Optimum parameters were studied giving to specify the chemical and physical parameters. Two line manifold was used. The flow rate of 1.3 and 1.5 mL/min was used, 35µL sample volume no.$_1$ and sample volume no.$_2$, linear dynamic range extend from 0.05-7, 0.05-7, 0.1-10 and 0.1-10 mMol.l^{-1} with correlation coefficient of 0.9933, 0.9964, 0.9984 and 0.9973 for HCl, H_2SO_4 , $HClO_4$ and tartaric acid respectively. Using t-test it was shown that there was no significant difference between two methods (adopted and classical method) for both acid at 95% confidence level.

Keywords: Laser diode fluorimeter , flow injection analysis , Oxonium ion

تقدير ايون الاوكسونيوم وتطبيقاته باخماد ضوء الفلورة المستمرة (جزيئة الفلورسين) باستخدام نظام الاخماد $I^- -IO_3^- -H_3O^+$

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الخلاصة

يقدم البحث طريقة سريعة ، مضبوطة وحساسة لتقدير ايون الاوكسونيوم (الهيدروكلوريك ، الكبريتيك، البريكلوريك وحامض الترتاريك). أستندت الطريقة على تحرير جزيئة اليود من خلال تفاعل $I^- -IO_3^- -H_3O^+$ وهذه الجزيئة المتحررة تتفاعل مع محلول الفلورسين مسببة أخماد ضوء الفلورة المستمرة عند التشعيع بطول موجي 405 نانومتر تم دراسة الظروف الفضلى لتشخيص المتغيرات الكيميائية والفيزيائية. تم استخدام وحدة متشعبة من خطين وبسرع جريان 1.3، 1.5 مل.دقيقة$^{-1}$ وحجم الانموذج للصمام الاول والثاني 35 مايكرولتر ، المدى الخطي الفعال يمتد من0.05−7,0.05−7, 0.1−10و 0.1−10 مللي مول.لتر$^{-1}$ وبمعامل ارتباط 0.9933,0.9964,0.9984 و 0.9973 لحامض الهيدروكلوريك، الكبريتيك ، البيركلوريك والترتاريك على التوالي. تم اجراء اختبار−t للمقارنة بين الطريقة المقترحة والطريقة القياسية ولوحظ انه لايوجد فرق جوهري بين الطريقتين عند مستوى ثقة 95%.

Introduction

Acids are essential substances in home, industry and the environment. 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

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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.

Experimental

A stock solution (0.01 $Mol.l^{-1}$) of fluorescein salt ($C_{20}H_{10}Na_2O_5$, M.Wt 376.27 $g.moL^{-1}$, 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,0.3 $Mol.l^{-1}$) was prepared by dissolving 24.90g in 500mL of distilled water and potassium iodate (KIO_3, M.Wt 214$g.mol^{-1}$, BDH,0.25 $Mol.l^{-1}$) 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,2 $Mol.l^{-1}$), sulphuric acid (98% w/w , 1.84 $g.ml^{-1}$, BDH,2 $Mol.l^{-1}$) and perchloric acid (85% w/w , 1.69 $g.ml^{-1}$,BDH,2 $Mol.l^{-1}$) 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 2 $Mol.l^{-1}$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, 0.1 $Mol.l^{-1}$) was prepared by dissolving 1.5009g in 100mL of distilled water.

Reagents and Chemicals

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 (50 mm (L), 50 mm (W), 50 mm (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 –2channel 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).

Methodology

The flow injection manifold reaction coupled with laser diode fluorimeter (blue purple 405nm-green 532nm) as shown in Figure-1A was used for the determination of oxonium ion throughout this work. The manifold reaction system is composed of two lines. The first line is the carrier stream (distilled water) at 1.30mL/min flow rate which lead to the injection valve no.$_1$ to carry oxonium ion sample segment (2m $Mol.l^{-1}$,4m $Mol.l^{-1}$HCl,35µL) followed by the departure of the sample segment from loop no.$_1$ until an entering loop no.$_2$ which transfer mixing solution (100m$Mol.l^{-1}I^-$-50m$Mol.l^-$

$^{1}IO_3^-$) (35µL) to mix with second line at 1.50mL/min (supplier of fluorescein sodium salt solution(5mMol.l^{-1})) at a Y-junction leading to reaction coil (Length 50cm,I.D,2mm) for completion of reaction i.e conversion of I^-&IO_3^- in acidic medium to free iodine and then to measuring cell to give quenching fluorescence response were measured using laser diode fluorimeter as shown in Figure-1B.

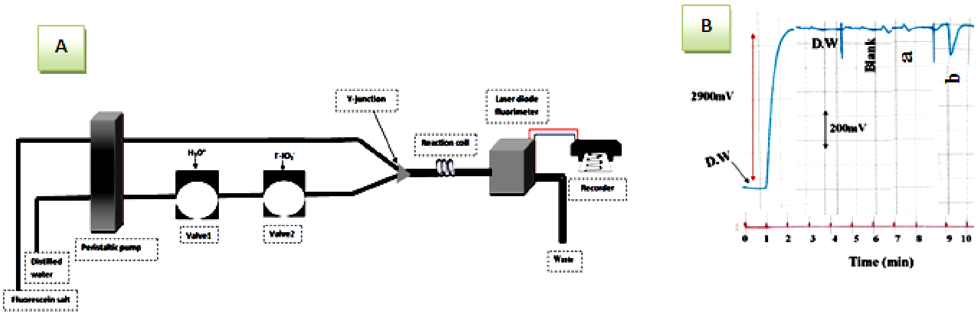
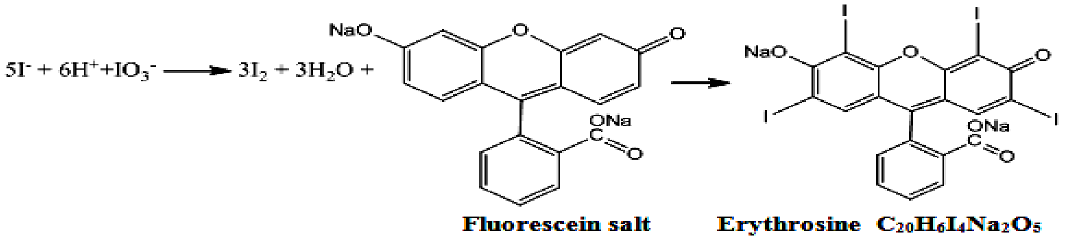


Figure 1- A- Schematic diagram of flow injection analysis coupled with laser diode fluorimeter used for the determination of oxonium ion B- Response profile of 5mMol.l^{-1} fluorescein salt solution using quenching System :a- 100mMol.l^{-1}I^--50mMol.l^{-1} IO_3^--2 mMol.l^{-1} HCl and b- 50mMol.l^{-1}I^--10mMol.l^{-1} IO_3^--4mMol.l^{-1} HCl

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



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

Variable optimization

A series of experiments were conducted to establish the conditions for the formation quenching fluorescence response with best working optimum reaction parameters (the chemical and physical parameters were investigated respectively).

Chemical variables effect

Fluorescein salt concentration

A series of the fluorescein salt solutions (0.005-7mMol.l^{-1}) at 1.50mL/min flow rate were prepared. A 4mMol.l^{-1} of hydrochloric acid was used with 27µL sample volume no.$_1$ and 50mMol.l^{-1}(I^-)-10mMol.l^{-1} (IO_3^-)at valve no.$_2$ (31µL) with flow rate 1.30mL/min using sequential open valves mode. Each measurement was repeated for three successive times. Table-1 was obtained explaining the increase in the continuous fluorescence with increases of fluorescein salt concentration and increases of quenching fluorescence response as shown in Figure-2.A.B followed by a stability in quenching fluorescence response at height concentration of fluorescein salt (i.e.,5mMol.l^{-1}) therefore 5mMol.l^{-1} was chosen as optimal concentration that give optimal fluorescence intensity with low consumption of reagent concentration.

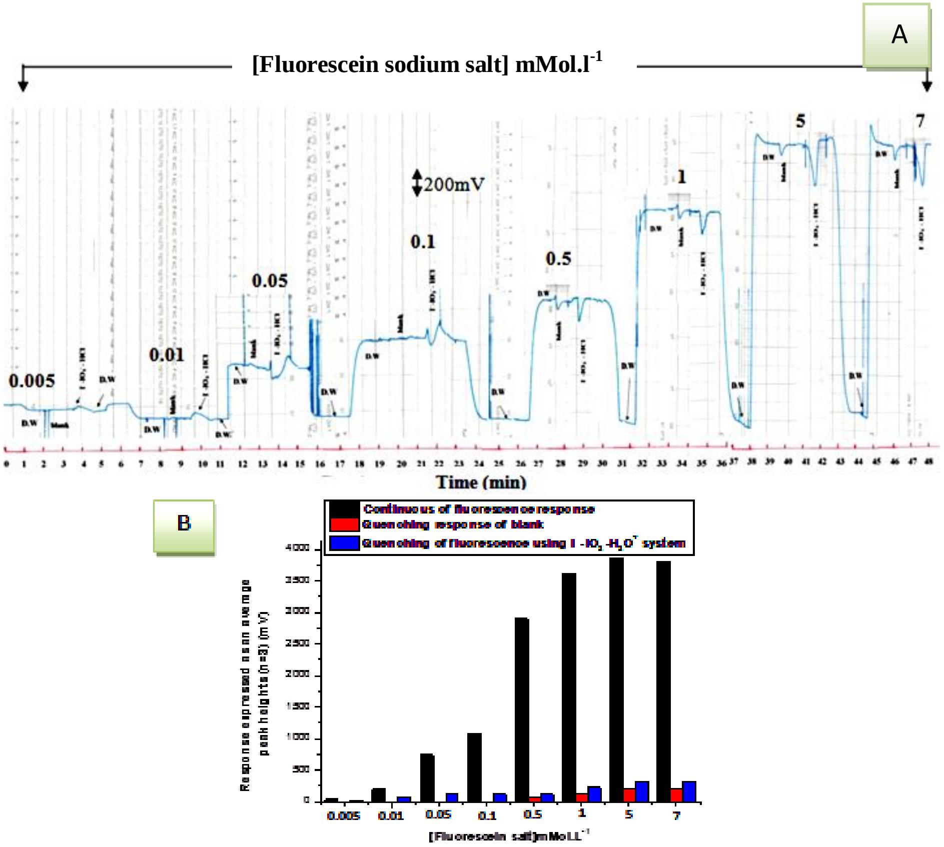


Figure 2- A- Response profile for eight concentrations of fluorescein salt solution B- Effect of fluorescein salt concentration on : response height of continuous fluorescence , quenching of fluorescence by : blank (I^--IO_3^-) and I^--IO_3^--H_3O^+ system

Table 1- Effect of fluorescein sodium salt concentration on continuous fluorescence

Concentration of fluorescein salt mMol.l^{-1}	**Continuous of fluorescence response (mV)**	**Response of 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	40±0.25	0±0.0	20±1.89	20	20
0.01	200±0.57	0±0.0	80±4.29	80	120
0.05	740±1.74	0±0.0	110±3.28	110	630
0.1	1080±2.48	0±0.0	120±5.17	120	960
0.5	2900±0.79	80±0.49	200±6.46	120	2700
1	3620±0.62	120±0.75	340±3.99	220	3280
5	3850±0.75	200±0.62	515±5.74	315	3335
7	3800±0.49	200±0.72	520±4.69	320	3280

Response of D.W= 0

Effect of iodide ion concentration with different concentration of iodate ion on quenching fluorescence response

The study was carried out using variable concentration of either iodide ion (10,30,50,100mMol.l^{-1}) and iodate ion (2,5,10,50, 100mMol.l^{-1}), using sequential open valves mode.Mixing solution (I^-&IO_3^-) injected at valve no.$_2$ (31µL) and HCl solution 4mMol.l^{-1} injected at valve no.$_1$ (27µL) with flow rate 1.30, 1.50mL/min of carrier stream and fluorescein sodium salt solution (5mMol.l^{-1}) respectively. Figure-3A shows the response profile of total quenched fluorescence versus concentration, while Figure-3B show the effect of either I^- and IO_3^- on quenched fluorescence response expressed as an average peak heights. All results tabulated in Table-2. It was noticed clearly that an increasing in peak height with increasing of iodide and iodate ion concentrations therefore, 100 mMol.l^{-1} I^- -50 mMol.l^{-1} IO_3^- was chosen as optimal concentrations of iodide and iodate ion to give optimal mixing solution to librated free iodine molecule which react with fluorescein molecule to quench the continuous fluorescence.

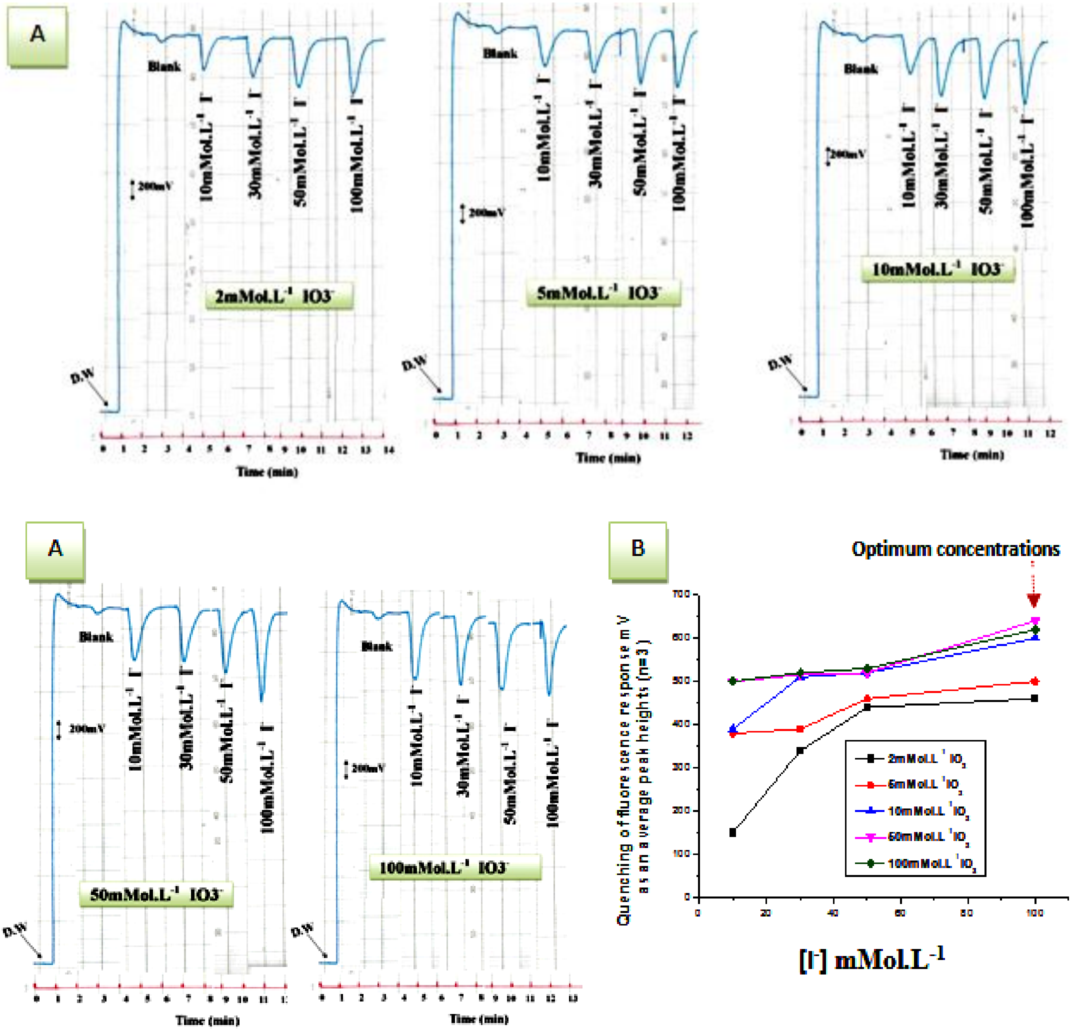


Figure 3- Effect of variable concentration of [I^-] using different concentration of IO_3^- on :

A- Sample of response profile using quenching fluorescence system I^--IO_3^-- H_3O^+

B-Quenching fluorescence response expressed as an average peak height in mV using 4mMol.l^{-1} HCl, 27µL.

Table 2- Variation of I^- concentration with different concentration of IO_3^- on quenched fluorescence response

Quenched fluorescence $\bar{y}_{Qi}$ (n=3)mV $\bar{y}_{Qi(mV)} \pm t_{0.05/2,\, n-1}\ \sigma_{n-1}/\sqrt{n}$				
Iodate ion concentration mMol.l^{-1}	**Iodide ion concentration mMol.l^{-1}**			
	10	**30**	**50**	**100**
2	150±3.78	340±3.13	440±4.37	460±3.13
5	380±6.24	390±7.60	460±5.17	500±3.99
10	390±4.47	510±8.97	520±7.97	600±6.58
50	500±5.71	515±4.47	520±4.69	640±5.74
100	500±4.97	520±3.28	530±3.78	620±7.18

Response of continuous fluorescence: 3850mV, Response of blank: 200mV

Physical parameter

Effect of flow rate

Using 4mMol.l^{-1}of HCl(27µL) - 100mMol.l^{-1}(I^-)- 50 mMol.l^{-1} (IO_3^-) (31µL) quenching system with fluorescein sodium salt solution 5mMol.l^{-1} and sequential open valves mode. A flow rates ranging from (0.2-1.75) and (0.25-2.00) mL/min for carrier stream and fluorescein solution respectively were assayed with the aim to evaluate their effect on the peak heights and repeatability of the analytical data. From Figure-4A, B and Table-3, it can be seen that there is no significant difference in the response height at low flow rate (<1.3 mL/min for carrier stream). This might be attributed to the dispersion (due to diffusion) leading to a larger volume of the sample segment which in turn to increase in the base width (Δt_b) and decrease in the quenching of fluorescence for continuous fluorescence intensity. While at higher flow rate (>1.3mL/min) there was a decrease in peak height, due to departure speed of quenching molecule (free iodine) at short time in addition to affect of dispersion (due to convection) and dilution as shown in Figure-4B. Therefore, a flow rate of 1.30, 1.50 mL/min for carrier stream and fluorescein salt solution respectively was chosen as optimum flow rate through this work.

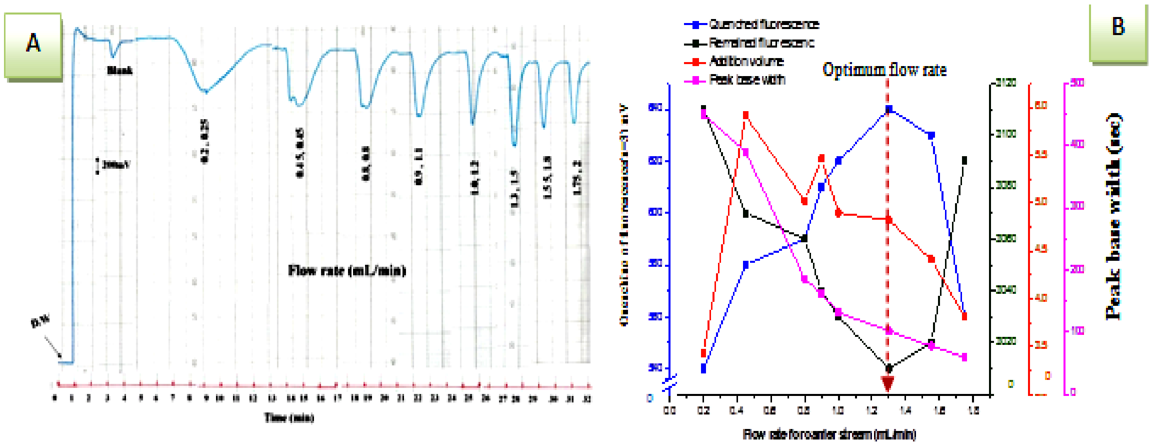


Figure 4- Effect of variation of flow rate (mL/min) on: A-Response profile, B-Quenched, remained of fluorescence,addition volume and peak base width in mV

Table 3- Effect of the variation of flow rate (mL/min) on the fluorescence response (total, quenched and remained) using I^--IO_3^--H_3O^+ system

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_{final} (mL)**	**Concentration in mMol.l^{-1} at flow cell**	**DF**
	Line$_1$	Line$_2$										
5	0.20	0.25	740	0.14	740±2.57	540	3110	450	114	3.433	0.031	127.15
10	0.45	0.45	780	0.34	780±6.59	580	3070	390	102	5.908	0.018	218.81
15	0.80	0.80	790	0.28	790±5.49	590	3060	186	84	5.018	0.022	185.85
20	0.90	1.10	810	0.16	810±3.22	610	3040	162	72	5.458	0.019	202.15
25	1.00	1.20	820	0.29	820±5.91	620	3030	132	60	4.898	0.022	181.41
30	1.30	1.50	840	0.22	840± 4.59	640	3010	102	36	4.818	0.022	178.44
35	1.55	1.80	830	0.21	830±4.33	630	3020	78	30	4.413	0.024	163.44
40	1.75	2.00	760	0.11	760±2.08	560	3090	60	24	3.808	0.028	141.04

Response of continuous fluorescence : 3850mV, Response of blank : 200mV
Δt_b (sec) : Time lapse for the segment of quenched fluorescence within measuring cell or peak base width
t: Departure time for sample segment from injection valve no.$_1$ to the measuring cell
V_{final}: Addition volume (mL) at each flow rate to obtain the final volume ,DF: Dilution factor at each flow rate

Effect of sample volume

Using variable volume of sample segment (4mMol.l^{-1} HCl) at injection valve no.$_1$ extended from 18-35µL, in addition to variable sample volume of valve no.$_2$ (18-43µL) were studied with sequential open valves mode. Using 5mMol.l^{-1} fluorescein sodium salt solution with 100mMol.l^{-1} (I^-) -50mMol.l^{-1} (IO_3^-) complement solution at flow rate 1.30 and 1.50 mL/min for carrier stream and fluorescein sodium salt solution respectively. All results tabulated in Table-4. It was noticed that an increase in sample volume no.$_1$ &no.$_2$ lead to increasing of quenched fluorescence response using sample volume larger than 35µL for both valves gave a nearly constant response as shown in Figure-5A, B , which can be probably attributed to continuation of the passage of carrier stream through the valves (i.e valve no.$_1$&valve no.$_2$) will cause an increase in the dispersion for the I_2 segment that cause a long time duration of sample segment in front of detector. Therefore; 35µL, 35µL were chosen as an optimal sample volume no.$_1$ and no.$_2$ respectively.

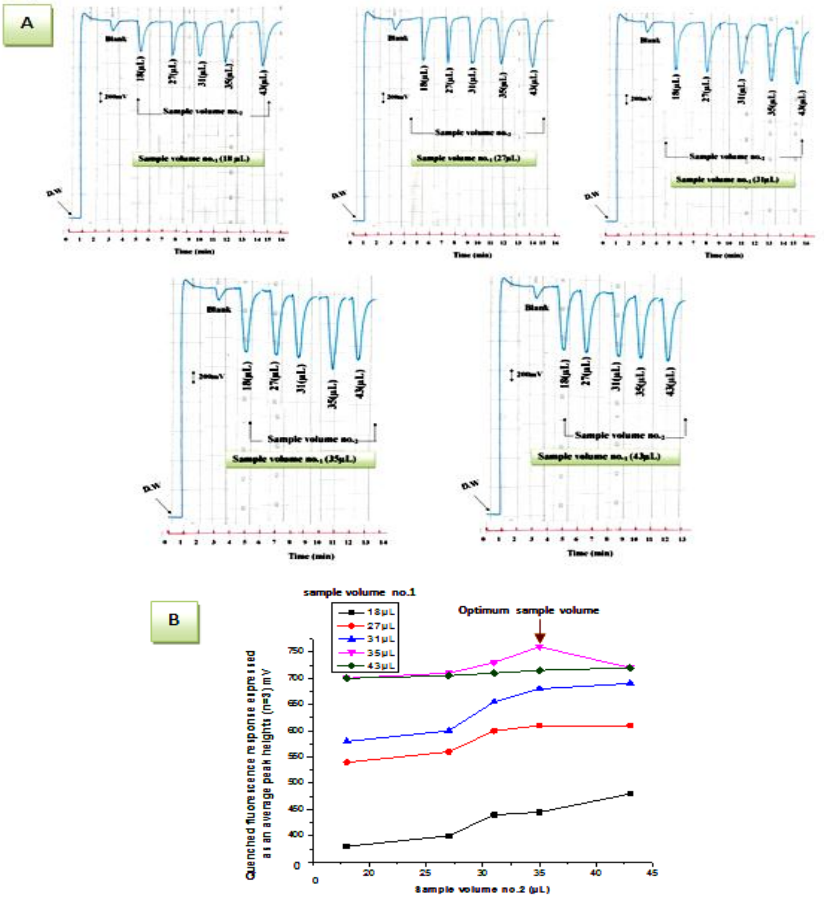


Figure 5- Effect of different sample volume no.$_1$& no.$_2$ on: A-Sample of response profile, B-Quenched fluorescence response expressed as an average peak heights (mV) using laser diode fluorimeter

Table 4- Variation of sample volume no.$_1$ & no.$_2$ on quenched fluorescence response using I^- - IO_3^- -H_3O^+ system

Sample volume no.$_1$ (µL)	**Sample volume no.$_2$ (µL)**				
Quenched fluorescence $\bar{y}_{Qi}$ (n=3)mV $\bar{y}_{Qi(mV)} \pm t_{0.05/2,\, n-1}\ \sigma_{n-1}/\sqrt{n}$					
18	18	27	31	35	43
	380±6.58	400±3.78	440±3.99	445±5.59	480±6.58
27	540±4.97	560±4.37	600±4.97	610±5.74	610±5.42
31	580±3.80	600±8.97	655±4.22	680±6.58	690±7.45
35	700±4.47	710±7.97	730±3.75	760±3.50	720±3.40
43	700±4.97	705±6.46	710±7.95	715±3.73	720±5.71

Response of continuous fluorescence: 3850mV, Response of blank: 200mV

Purge time & order of the addition of complementary chemicals used for the generation of iodine

The purge time of injected sample segment between injection valve no.$_1$ and valve no.$_2$ using sequential valves mode was studied at variable time lapse (2-40seconds) using the achieved parameters. Order of reaction using valve no.$_1$&valve no.$_2$ in sequence of H_3O^+ in valve no.$_1$ as a sample loop while(I^-,IO_3^-) in valve no.$_2$. This was regarded as mode no.$_1$ while otherwise is regarded as mode no.$_2$. Figure-6A, B shows the above explanation, also it indicate the sequence of carrying. Figure-6C shows different modes for two injection valve in the carrying stream line (i.e. injection &load) mode.

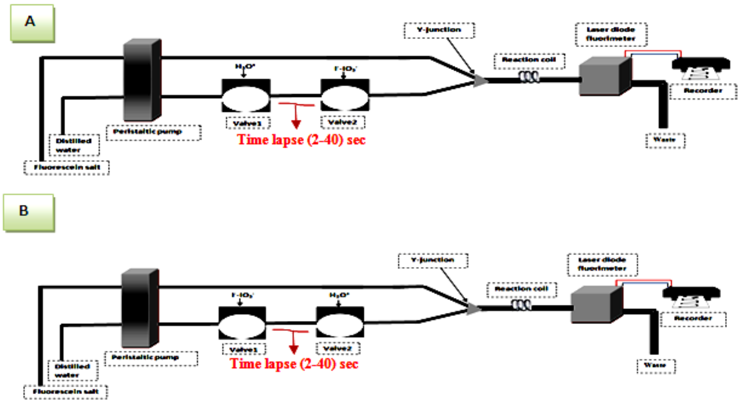


Figure 6- Flowgram of manifold design when using two different injection mode: A-Injected H_3O^+ at valve no$_1$& I^- - IO_3^- at valve no.$_2$ B- injected I^- - IO_3^- at valve no.$_1$& H_3O^+ at valve no.$_2$

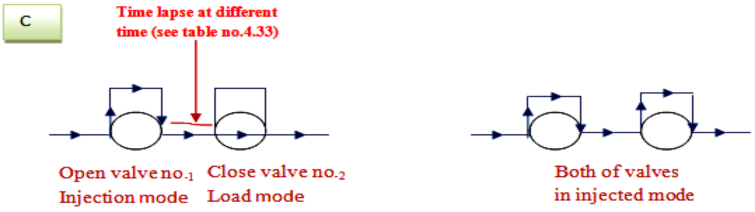


Figure 6C- Schematic flowgram of manifold system for carrying stream line using two valves

From this study, it comes to a conclusion that mode no.$_1$ is preferable to mode no.$_2$, which might be due to the obstacle of higher density of(I^-,IO_3^-) in valve no.$_2$, also the present valve no.$_1$ before valve no.$_2$ might slow down little bit the movement of liquids through the tubes. Table-5 summarizes the data obtained. Figure-7A shows the plot of the effect of variation for the allowed permissible time on response profile. It was noticed that there is an increase in the response with increasing the allowed permissible time for the sample injection up to 26seconds, then a decrease in the response obtained. This might be attributed to a destruction of flow as a result of elongated period of leaving the sample segment in the injection valve which leads to the slow movement of the iodine segment in the measuring cell. Therefore, 26seconds was chosen as the optimum allowed permissible time between two valves Figure-7B.

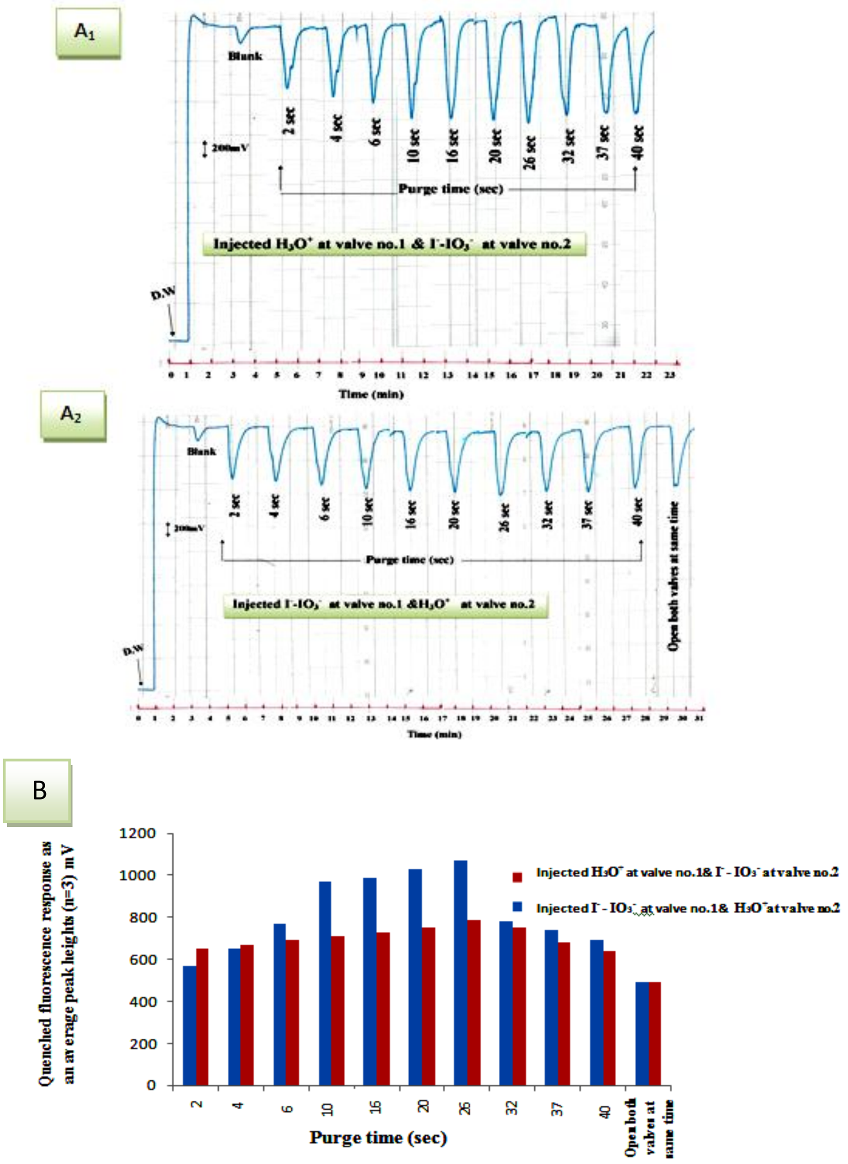


Figure 7-Effect of purge time with variation of injection mode on: A$_1$-Response profile when H_3O^+ injected at valve no$_1$& I^- - IO_3^- at valve no.$_2$ A$_2$-Response profile when injected I^- - IO_3^- at valve no.$_1$& H_3O^+ at valve no.$_2$. B-Quenched fluorescence response in mV using two injection mode

Table 5- Variation of purge time on the fluorescence response with replacement of H_3O^+ & I^--IO_3^- between two valves

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**
Valve no.$_1$ (H_3O^+)&valve no.$_2$(I^--IO_3^-)					
2	780	570	0.17	780±3.29	3020
4	860	650	0.35	860±7.48	2940
6	980	770	0.2	980±4.87	2820
10	1180	970	0.16	1180±4.69	2620
16	1200	990	0.19	1200±5.66	2600
20	1240	1030	0.32	1240±9.86	2560
26	1280	1070	0.13	1280±4.13	2520
32	1000	790	0.10	1000±2.48	2800
37	950	740	0.05	950±1.18	2850
40	900	690	0.21	900±4.69	2900
Valve no.$_1$ (I^--IO_3^-)&valve no.$_2$(H_3O^+)					
2	860	650	0.29	860±6.19	2940
4	880	670	0.16	880±3.49	2920
6	900	690	0.3	900±6.71	2900
10	920	710	0.28	920±6.39	2880
16	940	730	0.16	940±3.74	2860
20	960	750	0.11	960±2.62	2840
26	1000	790	0.15	1000±3.73	2800
32	960	750	0.21	960±5.01	2840
37	890	680	0.34	890±7.52	2910
40	850	640	0.17	850±3.59	2950
Open both valves at the same time	700	490	0.57	700±9.91	3100

Response of continuous fluorescence: 3800mV, Response of blank : 210mV

Reaction coil

Variable coil lengths comprises a volume of 0.235-1.325mL was used. The coil was connected in two positions, the firstly connected after injection valve no.$_2$ directly and the second position after Y-junction point as shown in Figure-8A,B with optimum parameters that were achieved in previous sections. Figure-9A shows type of response profile and Table-6 shows all the obtain results of coil length effect on the total, quenched and remained fluorescence response. It can be seen that there is no significant difference between responses with changeable reaction coil (various coil length) at any position which most probably attributed to the increase effect of dilution and dispersion on free iodine molecule, quenched fluorescence segment and distribution of I_2 species on a larger area that might give a longer time duration of I_2 in front of the detector. On this basis two lines manifold without additional coil was used.

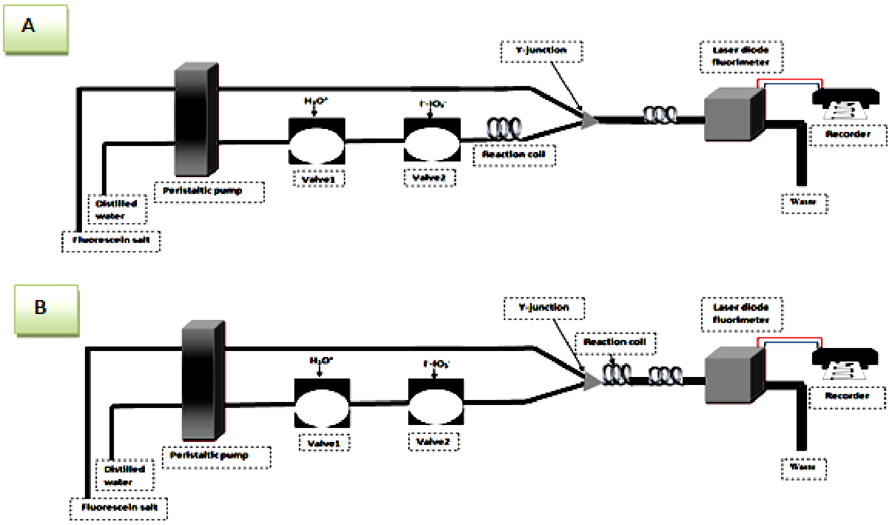


Figure 8- Flow diagram manifold system using reaction coil connected: A-after injection valve no.$_2$. B- after Y-junction point

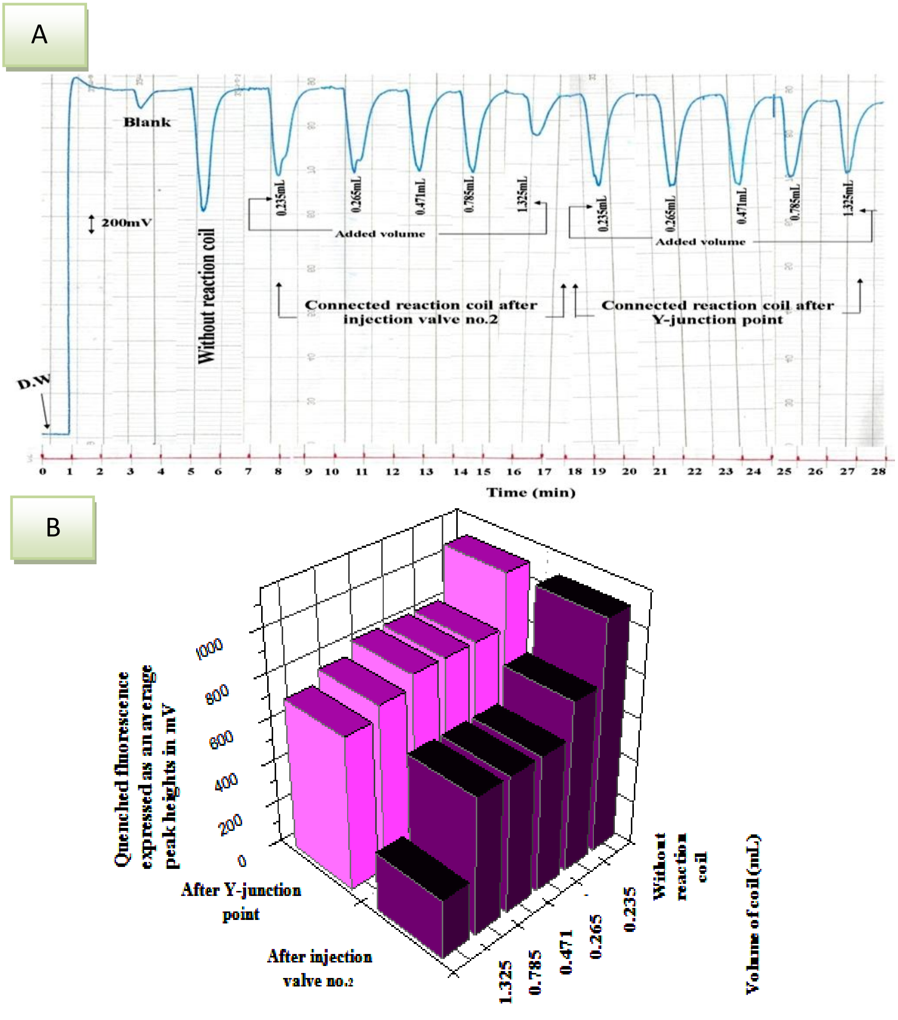


Figure 9-Effect of reaction coil on A-Response profile. B-Quenched fluorescence expressed as an average peak heights (n=3) in mV

Table 6- Effect of variation of coil length on total, quenched and remained fluorescence response for determination of oxonium ion

Coil volume (mL) (r(mm)) **Coil length (cm)**	**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**
Connected reaction coil after injection valve no.$_2$					
Without coil **Without coil**	1285	1075	0.13	1285±4.15	2515
0.235 (0.5) 30	1000	790	0.29	1000±7.20	2800
0.265 (0.75) 15	840	630	0.27	840±5.63	2960
0.471 (0.5) 60	840	630	0.28	840±5.84	2960
0.785 (0.5) 100	840	630	0.43	840±8.97	2960
1.325 (0.75) 75	480	270	0.50	480±5.96	3320
Connected reaction coil after Y-junction point					
0.235 (0.5) 30	1040	830	0.18	1040±4.65	2760
0.265 (0.75) 15	1040	830	0.15	1040±3.88	2760
0.471 (0.5) 60	1040	830	0.20	1040±5.17	2760
0.785 (0.5) 100	980	770	0.22	980±5.36	2820
1.325 (0.75) 75	920	710	0.22	920±5.03	2880

Response of continuous fluorescence: 3800mV, Response of blank: 210mV

Study of the variation of oxonium ion concentration on the total, quenched and remained fluorescence response and statistical parameters

After optimization the chemicals and physical parameters, the calibration curve of oxonium ion (HCl, H_2SO_4 , $HClO_4$ and tartaric acid) using laser diode fluorimeter were evaluated. A series (0.005-20)mMol.l^{-1} oxonium ion solutions were prepared as shown in Figure-10. Table-7 depicts all the results obtained using linear regression equation. This method compared with classical method using calibrated pH-meter at the same range of concentration. The limit of detection was 4µMol.l^{-1} for HCl & H_2SO_4 while 9µMol.l^{-1} for $HClO_4$ & tartaric acid. All results tabulated in Table-8.

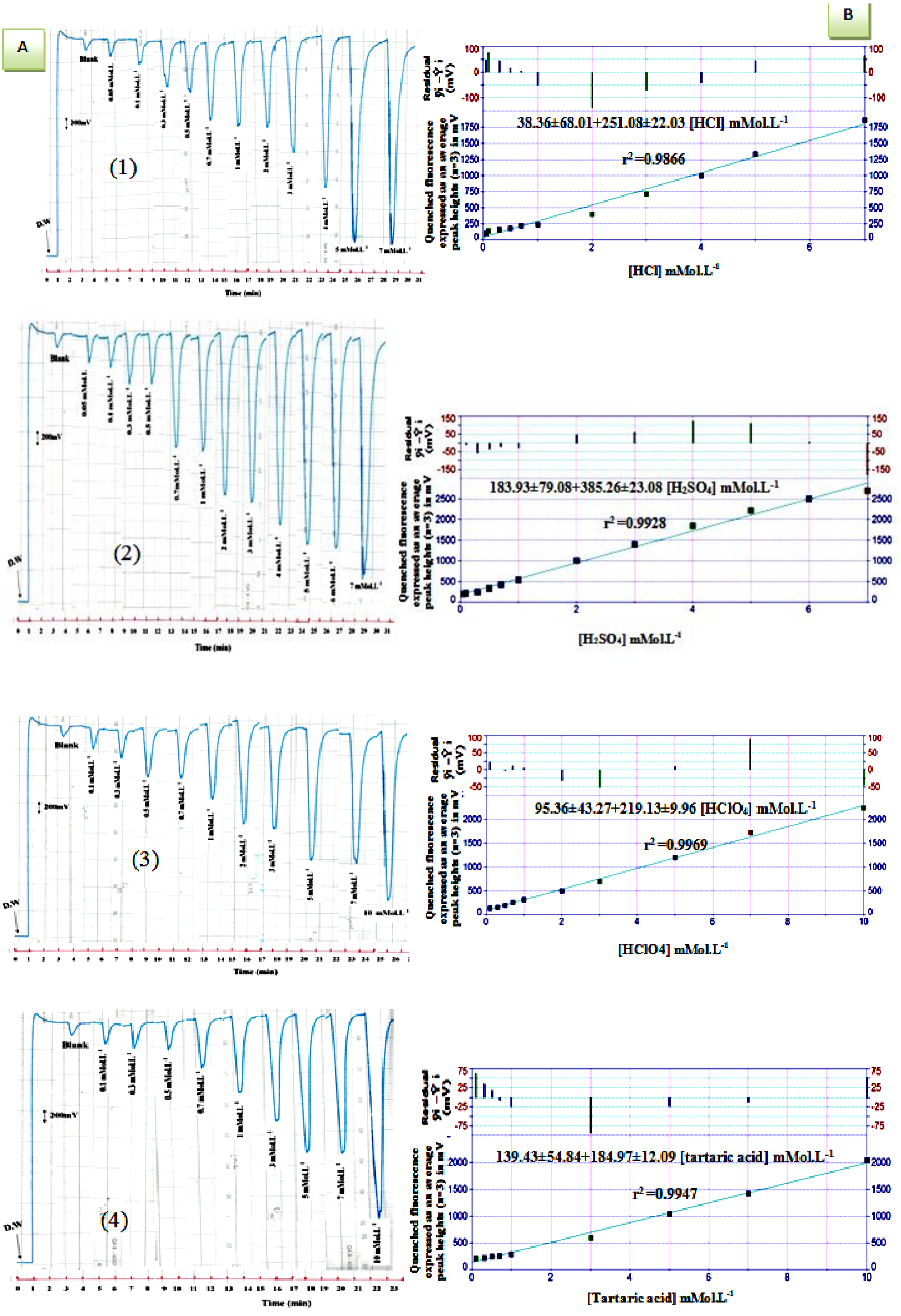


Figure 10- Calibration graph for the variation of oxonium ion concentration on:A-Response profile for 1- HCl,2-H_2SO_4,3-$HClO_4$, 4-Tartaric acid. B-Quenched fluorescence response for1-HCl,2-H_2SO_4,3- $HClO_4$, 4-Tartaric acid. Residual ($\bar{y}i$ – $\hat{Y}i$), $\bar{y}i$: practical value, $\hat{Y}i$: estimate value.

Table 7- 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**	**$\hat{y}(mV)=(a\pm S_at)+(b\pm S_bt)[H_3O^+]$ 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 $Ka=10^6$	0.005-20	n=11 0.05-7	Total quenching of fluorescence	238.36±68.01+251.08±22.03 [HCl]mMol.l^{-1}	0.9933 0.9866 98.66	2.262<< 25.78	
			Quenching of fluorescence	38.36±68.01+251.08±22.03 [HCl] mMol.l^{-1}			
			Remained of fluorescence	3561.64±68.01-251.08±22.03 [HCl] mMol.l^{-1}			
H_2SO_4 $Ka=10^3$		n=12 0.05-7	Total quenching of fluorescence	383.93±79.08+385.26±23.08 [H_2SO_4] mMol.l^{-1}	0.9964 0.9928 99.28	2.228<< 37.19	
			Quenching of fluorescence	183.93±79.08+385.26±23.08 [H_2SO_4] mMol.l^{-1}			
			Remained of fluorescence	3416.07±79.08-385.26±23.08 [H_2SO_4] mMol.l^{-1}			
$HClO_4$ $Ka=10^3$		n=10 0.1-10	Total quenching of fluorescence	295.36±43.27+219.13±9.96 [$HClO_4$] mMol.l^{-1}	0.9984 0.9969 99.69	2.306<< 50.74	
			Quenching of fluorescence	95.36±43.27+219.13±9.96 [$HClO_4$] mMol.l^{-1}			
			Remained of fluorescence	3504.64±43.27-219.13±9.96 [$HClO_4$] mMol.l^{-1}			
Tartaric acid $Ka_1=1.29x10^{-3}$ $Ka_2=3.98x10^{-5}$		n=9 0.1-10	Total quenching of fluorescence	339.43±54.84+184.97±12.09 [tartaric acid] mMol.l^{-1}	0.9973 0.9947 99.47	2.365<< 36.15	
			Quenching of fluorescence	139.43±54.84+184.97±12.09 [tartaric acid] mMol.l^{-1}			
			Remained of fluorescence	3460.57±54.84-184.97±12.09 [tartaric acid] mMol.l^{-1}			

ŷ: estimated response (mV) for (n=3) expressed as an average peak heights of linear equation 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.

From the outcome of the above studies that when dealing with high concentration of acid; a deviation from linearity occur and a decrease in correlation coefficient this might be due to the precipitate of fluorescein as an acid from its sodium salt. Any distorted profile of the energy transducer output response could be due to tiny particle of fluorescein free acid (that is insoluble in water or acid media) that affect the direction of the incident beam due to scattering effect.

Table 8-Calibration graph for determination of oxonium ion (HCl, H_2SO_4 $HClO_4$ and tartaric acid) by 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=9 0.005-10	110.26±33.50+30.03±7.96[HCl]mMol.l^{-1}	0.9587 0.9191 91.91	2.365<< 8.92	
H_2SO_4	n=9 0.005-10	110.09±31.07+39.93±7.38[H_2SO_4] mMol.l^{-1}	0.9792 0.9589 95.89	2.365<< 12.79	
$HClO_4$	n=10 0.01-10	116.00±29.30+27.14±7.33[$HClO_4$] mMol.l^{-1}	0.9493 0.9011 90.11	2.306<< 8.54	
Tartaric acid	n=10 0.01-10	102.08±27.04+29.22±6.76[Tartaric acid] mMol.l^{-1}	0.9619 0.9254 92.54	2.306<< 9.96	

$\hat{y}$: estimated response of pH at mV for n=3 expressed by 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.

Limit of detection

The limit of detection of oxonium ion was determined using the gradual dilution of the minimum concentration of the analyte in the calibration graph which was 0.01mMol.l^{-1} (HCl,H_2SO_4) and 0.05mMol.l^{-1} ($HClO_4$, tartaric acid) and based on the value of slope as tabulated in Table-9.

Table 9- Detection limit of oxonium ion using quenching system I^--IO_3^--H_3O^+

Type of acids	**Minimum* concentration (mMol.l^{-1})**	**Practical based on the gradual dilution for the minimum concentration**	**Theoretical based on the value of slope X=3S_B/slope**
HCl	0.01	12.78ng/sample	6.11ng/sample
H_2SO_4	0.01	34.30ng/sample	11.00ng/sample
$HClO_4$	0.05	175.88ng/sample	19.00ng/sample
Tartaric acid	0.05	262.66ng/sample	34.00ng/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**

Repeatability

The value of the relative standard deviation (RSD%) for the selected concentration of oxonium ion (7mMol.l^{-1}) was studied. A repeated measurements for eight successive injections were measured. Table 10 shows the arithmetic's, which shows that the percentage relative standard deviation was less than 1%. This low percentage indicate a reliable measurement can be achieved using this method. The kind of the response profile is shown in Figure-11.

Table 10-Repeatability of oxonium ion (HCl,H_2SO_4,$HClO_4$ and tartaric acid) at parameters via average of total and quenched fluorescence expressed as an average peak heights (n=8) in mV

Type of acid	**Average of total quenched fluorescence expressed as an average peak heights $\bar{y}_i$ in mV**	**Quenched fluorescence $\bar{y}_{Qi}$ (n=8)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	2030	1830	0.24	2030±4.07
H_2SO_4	2672	2472	0.15	2672±3.35
$HClO_4$	1700	1500	0.35	1700±4.98
Tartaric acid	1410	1210	0.60	1410±7.07

Response of continuous fluorescence=3800 mV , Response of blank : 200mV, $t_{0.05/2,\,7}$=2.365
Number of injection = 8

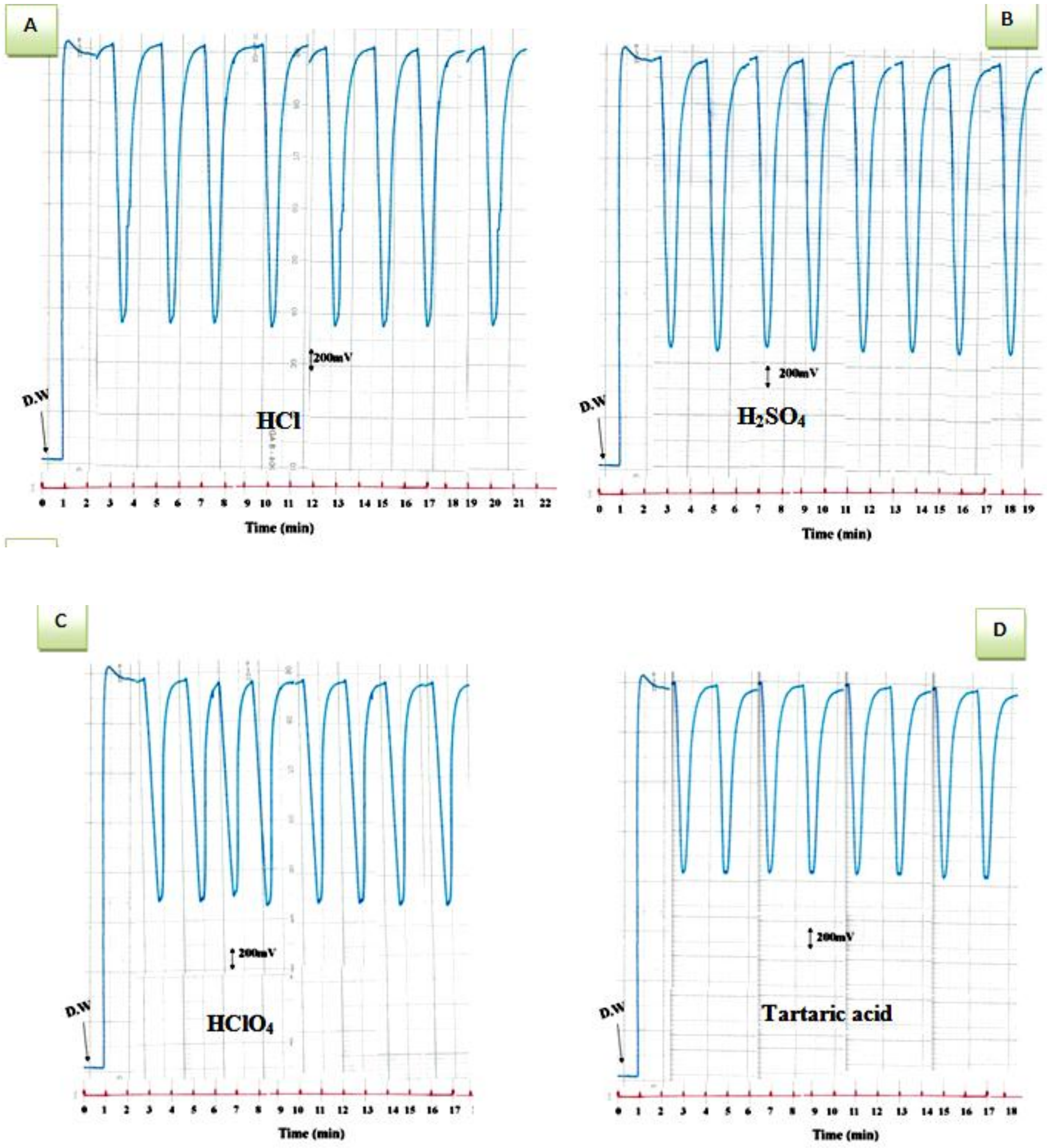


Figure 11- Response profile for eight successive repeated measurement of oxonium ion : A- HCl , B-H_2SO_4 , C-$HClO_4$ and D- tartaric acid

Application

Two methods were used for the determination of two different samples of acids (H_2SO_4, 98%, Loba Chemie-India & tartaric acid, Thomas Baker-India). The firstly methods was using laser diode fluorimeter while the second method was used the classical method (pH-meter). A series of solutions were prepared of each sample by transferring 1.5mL (50mMol.l^{-1}) to each of the five volumetric flasks (25mL), followed by the addition of 0 , 0.5 , 1 , 1.5 , 2mL from 50mMol.l^{-1} standard solution in order to have the concentration range from (0-4 mMol.l^{-1}). Figure-12A_1, B_1 shows response profile for this study and A_2, B_2, C and D standard addition calibration graph using two methods. Table-11 tabulated the summary of results for standard addition methods from the two samples.

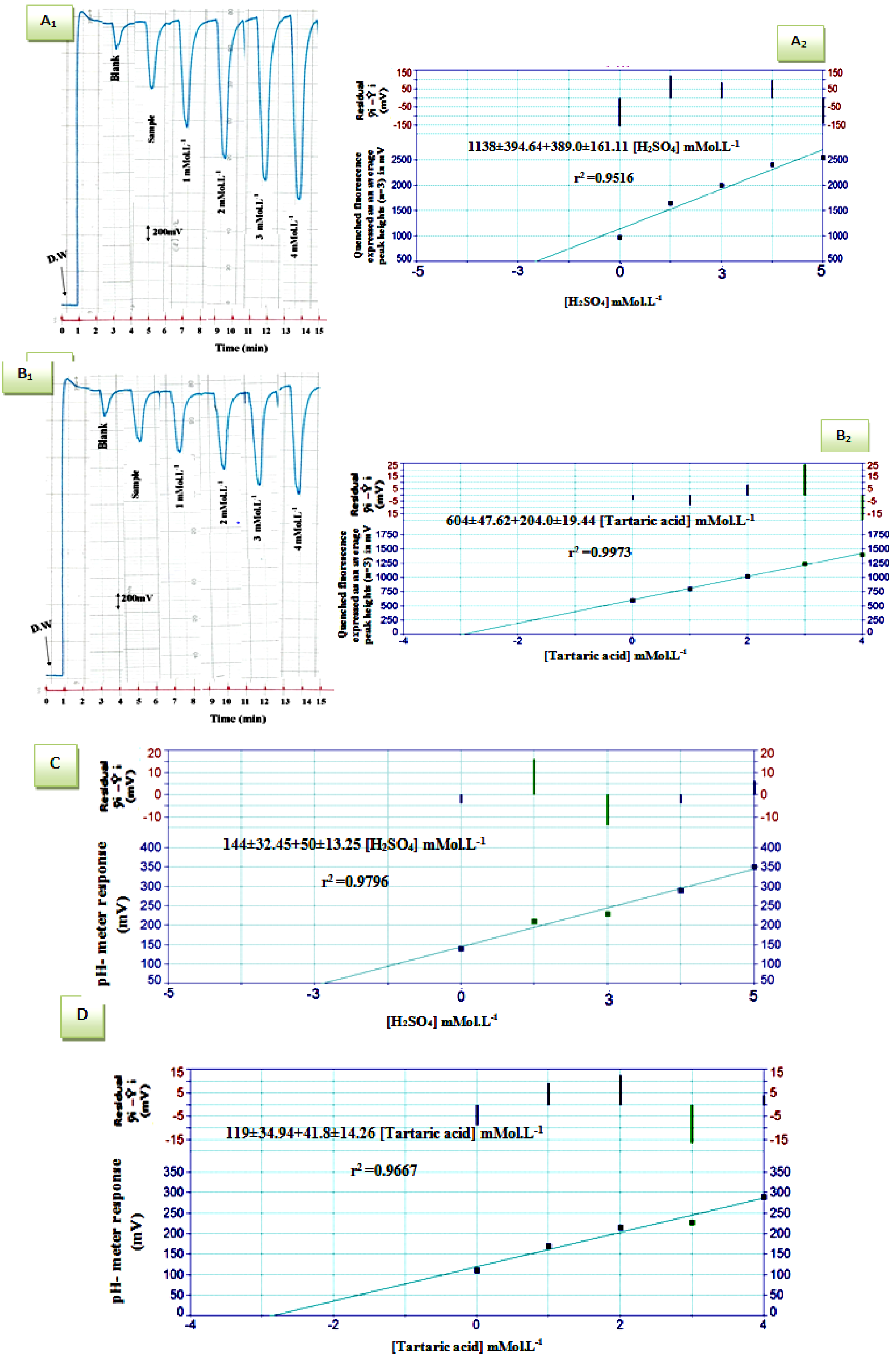
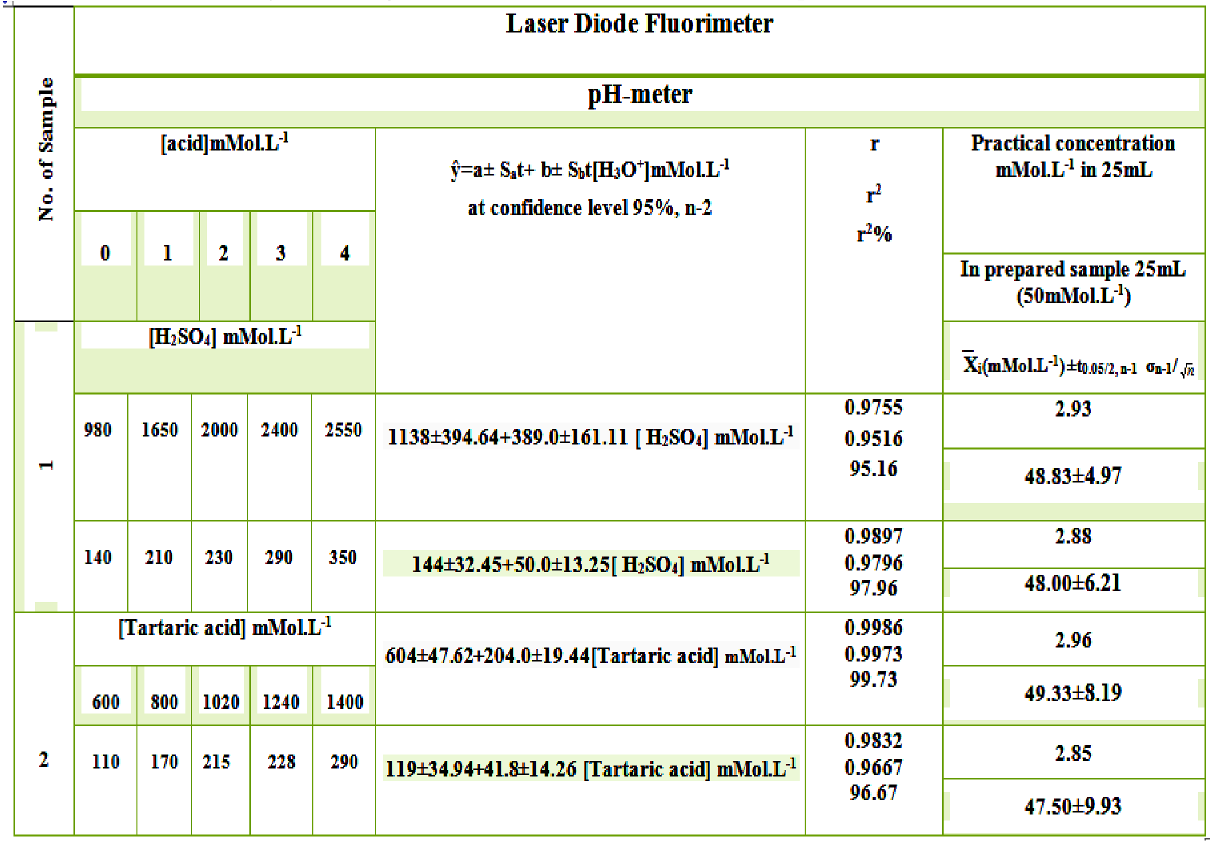


Figure 12- Sample response profile (A_1- H_2SO_4 , B_1-tartaric acid) with standard addition calibration graph (A_2-H_2SO_4 , B_2-tartaric acid) using laser diode fluorimeter and (C-H_2SO_4 , D-tartaric acid) using pH-meter.

Table 11-Summary of results by standard additions method for the determination of oxonium ion by fluorescence system using laser diode fluorimeter method and pH-meter method



No. of Sample	[acid]mMol.L^{-1} 0	1	2	3	4	$\hat{y}=a\pm S_a t+b\pm S_b t[H_3O^+]$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})$\pm t_{0.05/2,n-1}\,\sigma_{n-1}/\sqrt{n}$
	Laser Diode Fluorimeter							
1	[H_2SO_4] mMol.L^{-1}							
	980	1650	2000	2400	2550	1138±394.64+389.0±161.11 [H_2SO_4] mMol.L^{-1}	0.9755 0.9516 95.16	2.93 48.83±4.97
	pH-meter							
	140	210	230	290	350	144±32.45+50.0±13.25[H_2SO_4] mMol.L^{-1}	0.9897 0.9796 97.96	2.88 48.00±6.21
2	[Tartaric acid] mMol.L^{-1}							
	600	800	1020	1240	1400	604±47.62+204.0±19.44[Tartaric acid] mMol.L^{-1}	0.9986 0.9973 99.73	2.96 49.33±8.19
	110	170	215	228	290	119±34.94+41.8±14.26 [Tartaric acid] mMol.L^{-1}	0.9832 0.9667 96.67	2.85 47.50±9.93

$\hat{y}$: Estimated value (mV for laser diode fluorimeter and 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, $t_{0.05/2,\ 2=4.303}$

Individual paired t-test [17, 18] was used in order to compare developed method with the classical method for both samples. The results as shown in Table-12 indicated clearly that there were no significant difference between two methods for both acid at 95% confidence level since the calculated t-value is less than critical tabulated t-value.

Table 12-Paired t-test for the comparison between two methods using H_2SO_4 & tartaric acid as a samples

<table>
<tr><th rowspan="3">Type of sample</th><th colspan="4">Developed method (Laser Diode Fluorimeter) quenched of fluorescence</th></tr>
<tr><th colspan="4">Classical method (pH-mesurement in mV) directly measurement of $[H_3O^+]$</th></tr>
<tr><th>Practical value ($\bar{X}$) in mMol.l^{-1} for n=3 , μ =50mMol.l^{-1}
$\bar{X}_i$(mMol.L^{-1})$\pm t_{0.05/2,n-1}\,\sigma_{n-1}/\sqrt{n}$</th><th>$\sigma_{n-1}$</th><th>Paired t-test $(\bar{x}-\mu)\sqrt{n}/\sigma_{n-1}$</th><th>$t_{tab}$ ($t_{0.025,2}$) at 95% confidence level</th></tr>
<tr><td rowspan="2">**H_2SO_4**</td><td>48.83± 4.97</td><td>2.00</td><td colspan="2">|-1.01|<<4.303</td></tr>
<tr><td>48.00± 6.21</td><td>2.49</td><td colspan="2">|-1.39|<<4.303</td></tr>
<tr><td rowspan="2">**Tartaric acid**</td><td>49.33± 8.19</td><td>3.29</td><td colspan="2">|-0.35|<<4.303</td></tr>
<tr><td>47.50± 9.93</td><td>3.99</td><td colspan="2">|-1.09|<<4.303</td></tr>
</table>

μ: Theoretical value in mMol.l^{-1} , σ_{n-1} : Standard deviation , n= number of measured

Conclusion

The proposed method is simple and rapid with high sensitivity for the determination of oxonium ion (HCl,H_2SO_4,$HClO_4$ and tartaric acid) using quenched fluorescence system I^--IO_3^--H_3O^+ by liberation of iodine which react with fluorescein salt solution using two line manifold system causing to quench the fluorescence light (continuous fluorescence) when irradiated by laser source at 405nm. 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. Using t-test it was shown that there was no significant difference between two methods (laser diode fluorimeter and pH-meter) for both acid at 95% confidence level.

Acknowledgement

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