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Determination of oxonium ion using laser diode fluorimeter : (Blue purple(405 nm)-Green (532nm) using photodiode at 90^0 as a detector and I^- -IO_3^- -H_3O^+ system for quenching of fluorescence

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Abstract

A newly developed sensitive, fast and accurate analytical method characterized by a sensitive, fast and accuracy for the determination of oxonium ion by laser diode fluorimeter-flow injection analysis. The method is based on the libration of free iodine causing to quench the fluorescence light (continuous fluorescence from fluorescien molecule (free acid)) when irradiated by laser source at 405nm. Chemical and physical parameters were studied and optimized. The optimum parameters were 1.3mL/min using fluorescien (free acid) as a carrier stream, 35µL sample volume at valve no.$_1$ and 39µL at valve no.$_2$ and opened valves sequentially. Data treatment shows that linear range 0.002-0.1mMol.L^{-1},0.01-0.2mMol.L^{-1},0.002-0.5mMol.L^{-1},0.002-0.7mMol.L^{-1} for HCl, H_2SO_4,$HClO_4$ and tartaric acid respectively, with correlation coefficient r= 0.9562, 0.9976. 0.9755 and 0.9579. The limit of detection (S/N=3) 1.28ng/sample, 17.16ng/sample, 3.52ng/sample and 5.25ng/sample for HCl, H_2SO_4,$HClO_4$ and tartaric acid respectively from the stepwise dilution for minimum concentration of lowest concentration in linear dynamic range of the calibration gragh. RSD% for the repeatability (n=8) was <1% for the determination of HCl, H_2SO_4,$HClO_4$ and tartaric acid with concentration 0.01mMol.L^{-1} . The comparison was made between the newly developed method analysis with the classical method (pH-meter) using the standard addition method via the use of paired t-test was studied at two different paths .First path a comparison between two methods of analysis and second path between the two different acids. It was noticed that there was no significant difference between different acids and different methods at 95% confidence interval level.

Keywords: Laser diode fluorimeter , flow injection analysis

تقدير ايون الاوكسونيوم باستخدام محلل الحقن الجرياني فلوروميتر بثنائيات وصلة ليزرية :(ازرق-بنفسجي405نانومتر)-الاخضر(532نانومتر) باستخدام ثنائي وصلة فوتوني بزاوية 90^0 - I^- - IO_3^- كمتحسس لنظام اخماد الفلورسين H_3O^+

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الخلاصة

طورت طريقة جديدة تميزت بالحساسية والسرعة والدقة لتقدير ايون الاوكسونيوم بواسطة استخدام محلل الحقن الجرياني- فلوروميتير بثنائيات وصلة ليزري.استندت الطريقة على تحرير اليود مسببا أخماد ضوء الفلورة

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(الفلورة المستمرة من جزيئة الفلورسين الحامضية عند التشعيع بلمصدر الليزري بطول موجي 405نانومتر). تم دراسة المتغيرات الكيميائية والفيزيائية .حيث تم أختيار سرعة جريان مثلى1.3ملليتر/دقيقة باستخدام محلول الفلورسين كتيار ناقل وحجم انموذج 35 مايكروليتر لصمام الحقن الاول و39مايكروليتر لصمام الحقن الثاني.أظهرت نتائج معالجة البيانات ان مدى الخطية لحامض الهيدروكلوريك،الكبريتيك ،البريكلوريك والترتاريك هي 0.002−0.1 مللي مول لتر$^{-1}$،0.01−0.2 مللي مول لتر$^{-1}$، 0.002−0.5مللي مول لتر$^{-1}$ و0.002− 0.7 مللي مول لتر$^{-1}$ على التوالي بمعامل ارتباط (r) = 0.9562، 0.9976، 0.9755و 0.9579 على التوالي. حد الكشف 1.28نغم/ انموذج، 17.16 نغم/ انموذج،3.52 نغم/ انموذج و5.25نغم/ انموذج من التخفيف التدريجي لاقل تركيز في منحني المعايرة. الانحراف القياسي النسبي لتركيز 0.01 مللي مول لتر$^{-1}$ اقل من 1% بتكرارية لثمان مرات . اجريت المقارنة بين الطريقة المستحدثة والطريقة التقليدية (pH−meter) باستخدام الاضافات القياسية بوساطة اجراء اختبار t المزدوج بمسارين المسار الاول على اساس الاختلاف بين نوع الطريقة المستخدمة في التحليل والمسار الثاني استنادا على اختلاف مصدر الحامض ولوحظ من النتائج انه لايوجد فرق جوهري بين الطريقتين وبين مصدر الحامض المستخدم عند مستوى قناعة 95%.

Introduction

Acids play important roles in the human body. Inorganic acids are of prime importance in the chemical and metal industries, they are used as raw materials in the manufacture of a wide range of chemicals, as well as refining, electrolysis and extraction in chemical processes[1-6]. Hydrochloric acid is present in the stomach this aids in digestion by breaking down large and complex food molecules[7]. Sulphuric acid is used as a reagent rather than an ingredient. The largest single sulphuric acid consumer by far is the fertilities industry[8]. Perchloric acid is one of the most proven materials for etching of liquid crystal displays and critical electronics applications as well as one extraction and has unique properties in analytical chemistry[9]. Tartaric acid also has several applications for industrial use. The acid has been observes to chelate metal ions such as calcium and magnesium, therefore, the acid has severed in the farming and metal industries as a chelating agent for comlexing micronutrients in soil fertilizer and for cleaning metal surfaces consisting of aluminum, copper, iron and alloys of the metals, respectively[10-11].Many methods have been reported for the determination and study of oxonium ion [12-16].Fluorescien is a synthetic organic compound available as a dark orange/red powder slightly soluble in water and alcohol.It is widely used as a fluorescent tracer for many applications .The emission spectrum of fluorescien overlaps extensively the absorption of tetramethyl rhodamine, witch is a related strongly fluorescent dye, making this pair very suitable for energy transfer experiments to determine distances within and between labeled macromolecules.Flourescien in aqueous solutions occurs in cationic, neutral, anionic and dianionic forms making its absorption and fluorescence properties strongly pH dependent[17-20]. .The aim of this work is to present more sensitive, faster and simpler method for the determination of oxonium ion based on librated of free iodine by reaction between oxonium ion with mixture of iodide and iodate ions to formation quenching system for the fluorescien free acid solution (as continuous fluorescence) to quench light of fluorescence.

Experimental

Reagents and chemicals

A standard solution of sodium hydroxide (NaOH, M.Wt 40.0000 g.mol^{-1} , Fluka, 0.01Mol.L^{-1}) was prepared by dissolving 0.20g in 500 ml distilled water . A stock solution (0.01 Mol.L^{-1}) of fluorescien free acid ($C_{20}H_{12}O_5$, M.Wt 332.31 g.moL^{-1} , BDH) was prepared by dissolving 0.8307g in 250 ml of 0.01Mol.L^{-1} sodium hydroxide . 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.Wt 214g.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.

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(L)mm, 50(W)mm, 50(D)mm) 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 – 2 channels 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 diagram shown in figure-1.A which is composed of one line figure-1.A supply with fluorescien solution (free acid) (0.1 mMol.L^{-1}, pH=6.7) as a carrier stream that give a constant & continuous emission of fluorescence light when irradiated with 405nm laser beam as shown in fig.1.B. This line passes through two valves, where the acidic medium (i.e. sulphuric acid, 0.1 mMol.L^{-1}) inject at valve no.$_1$ with sample segment 35µL and the complement solution (i.e. iodide and iodate) inject at valve no.$_2$, 35µL at flow rate 1.3mL/min leading to librated free iodine which react with fluorescien solution causing to quench the fluorescence light, this might probably attributed to the consumption of fluorescien and the formation of iodine derivative of fluorescien molecule (i.e.Erythrosine)[20] according to the following scheme 1.

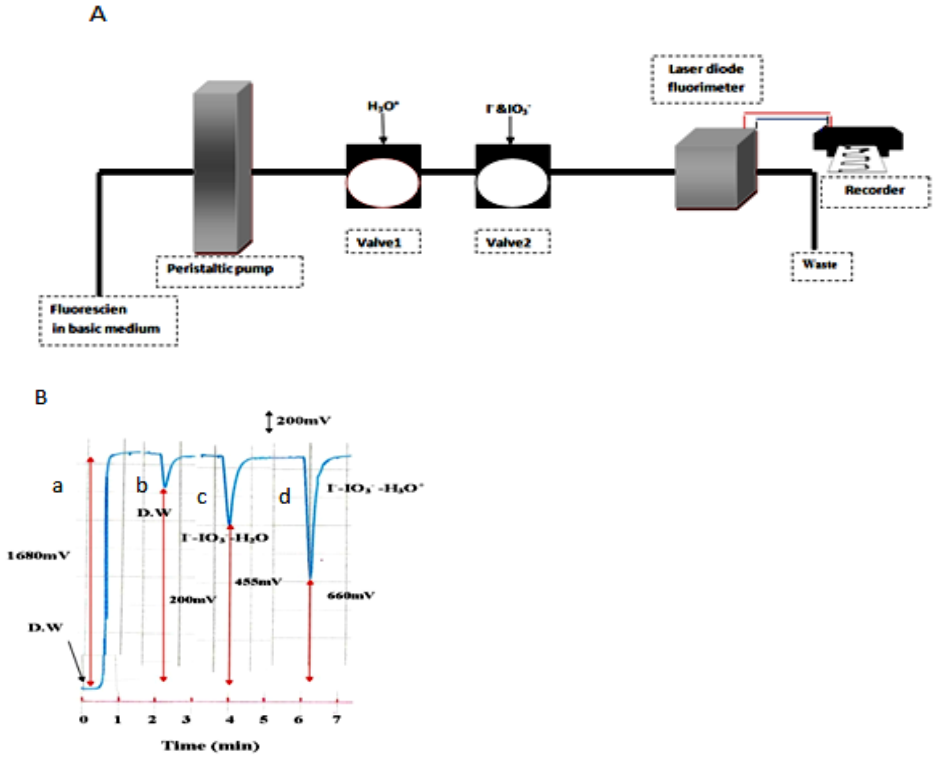
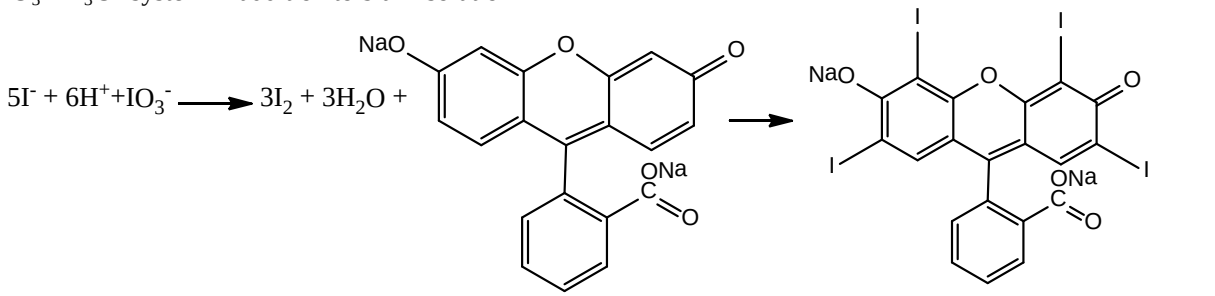


Figure 1- A- Flowgram of a manifold design consist of one line

B-Response profile using of 0.1 mMol.L^{-1} of fluorescien solution as a carrier stream a:total continuous response of fluorescence quenching by effect of b: D.W,c: I^- -IO_3^- -H_2O system (blank) and d: I^- -IO_3^- -H_3O^+ system in addition to blank solution



Fluorescien (free acid) in basic medium **Erythrosine $C_{20}H_6I_4Na_2O_5$**

Scheme 1-Proposed mechanism of the reaction between fluorescien and I_2 To illustrate the ability of librated iodine released from I^- -IO_3^- -H_3O^+ system to quench fluorescence of fluorescien solution.

1-Inject D.W in both valves

A response as shown in fig.1 (part b); to obtain the effect of water in quench fluorescence is done by subtracting the effect of D.W from total fluorescence

$D.W_{effect}$ = Total continuous response of fluorescence –Quenched response due to D.W i.e

$D.W_{effect} = F_T - Q_{D.W}$

= 1680-200 = 1480mV(remaineder of fluorescence)

2- Leaving valve no.$_1$(V_1) closed while injecting I^- &IO_3^- in the second valve(V_2). A response shown in fig.1(part c).

Effect of I^-, IO_3^-&H_2O = Total continuous response of fluorescence

–Response obtained from complementary chemical necessary for completion of reaction

= 1680-455 = 1225mV(remaineder of fluorescence)

Effect of I^- &IO_3^- only = Response obtained from the injection of I^- ,IO_3^- &H_2O–Quench response due to the effect of D.W= 455-200 = 255 mV

3-Injection of H_3O^+ in valve no.$_1$ (V_1) while injecting the rest of complimentary chemical to release I_2 in the valve no.$_2$(V_2) ; the following response as shown in fig.1(part d) was obtained.

To obtain the effect of I_2 in fluorescence quenching by subtracting the response of blank (i.e. I^- ,IO_3^-&H_2O) from the total response from I_2 to obtain the effect of I_2 only

Effect of I_2 = Total response of I_2 in the presence of blank -response of blank= 660-455 = 205 mV

Effect of I_2(i.e. I^- -IO_3^- - H_3O^+ system) : subtracting the I_2 response from total fluorescence to obtain the effect of I_2 in the fluorescence quenching which is followed in all measurements.

Effect of I_2 only= Total fluorescence – quench response due to the effect of I_2 only = 1680-205 = 1475mV

In all steps

To obtain the remainder of fluorescence is done from subtracting the total continuous response of fluorescence from the quenched response obtained by the effect of D.W or I^- -IO_3^- - H_2O system or by the effect of I_2 from I^- -IO_3^- - H_3O^+ system (which followed in all measurements).

Remained of fluorescence =Total continuous fluorescence –Total response from I_2& blank

= 1680 – 660 = 1020 mV

Study of the optimum parameters

The flow injection manifold system as shown in figure-1.A was investigated in the relation of chemical and physical variables, in order to obtain optimum conditions for the effect of I_2 in fluorescence quenching. They were optimized by making all variables constant and varying one at a time i.e. fixed variable optimization.

Variation of chemical parameters

A study was carried out to determine the optimum concentration of the reactant involved in the fluorescence quenching system

Effect of fluorescien (free acid) concentration

The study was carried out using a series of solutions by different concentration of fluorescien (0.01-1 mMol.L^{-1}) as a carrier stream at flow rate 1.3mL/min using close valves mode. A constant continuous fluorescence was obtained Figure-2 and table1 summarizes the obtain results ; it can be shows that an increase in the fluorescien concentration leads to an increase in the continuous fluorescence intensity causing a constant and continuous response (i.e.; steady response) due to the irradiation of fluorescien molecule by laser diode at λ_{max}=405nm with ≈ ≤ 100 mW but not enough energy to break the fluorophore molecule, at the same time transition it from ground state (S_o) to the excited level (S_1), followed by the fluorescient molecule return to the stable state ,fluorescent light will be librated reaching to 0.5mMol.L^{-1} .Using higher concentration leads to have deformed response profile and might cause a saturation of electronic detecting system used in the instrument. Therefore; 0.5 mMol.L^{-1}fluorescien concentration was chosen as the optimum concentration that used for future experiments.

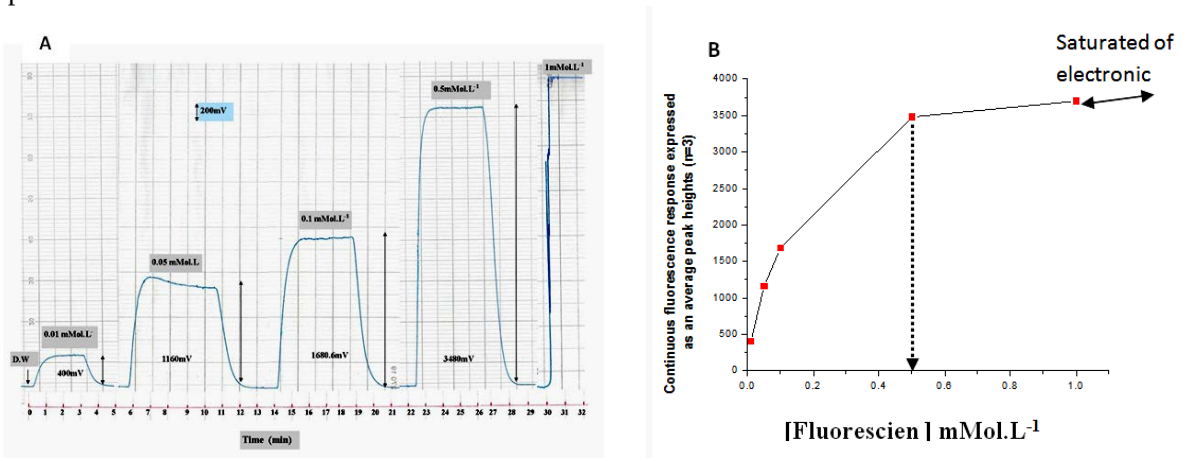


Figure 2-A- Fluorescence response profile versus time for the variation of fluorescien concentration
B-Relation between fluorescien concentration with continuous of fluorescence response expressed as an average peak heights (n=3)

Table 1-Effect of fluorescien concentration on continuous of fluorescence response expressed as an average peak heights $\bar{y}_i$ (n=3)

Concentration of fluorescien mMol.L^{-1}	**pH**	**Continuous of fluorescence response expressed as an average peak heights (n=3) $\bar{y}_i$ in mV**	**RSD%**	**Confidence interval of the average response (95% confidence level) $\bar{y}_i \pm t_{0.05/2,\, n-1}\ \sigma_{n-1}/\sqrt{n}$**
0.01	4.7	400	0	400±0
0.05	6.4	1160	0.06	1160±1.73
0.1	6.7	1680	0.07	1680.6±2.92
0.5	7.3	3480	0	3480±0
1	9.6	saturated	saturated	Saturated

Effect of sodium hydroxide concentration

A constant fluorescien concentration (0.5 mMol.L^{-1}) was prepared at variable concentration of NaOH ranging from (0.0005-0.4) Mol.L^{-1} . The pH of which was measured and found to be as follows 7.3, 12.1, 12.4, 12.8 and 13.2. Fig.3 and table 2 shows these responses versus variable NaOH concentration of the prepared above solution. A decease in fluorescence intensity was quite clear at higher pH value (pH >7.3) , and this might be attributed to the quenching effect of NaOH. Therefore , it was decided that minimum NaOH concentration (0.0005Mol.L^{-1}, pH=7.3) was the most suitable concentration at compromise for have a maximum fluorescence intensity & minimize the consumption of H_3O^+ by remaining it any present from NaOH

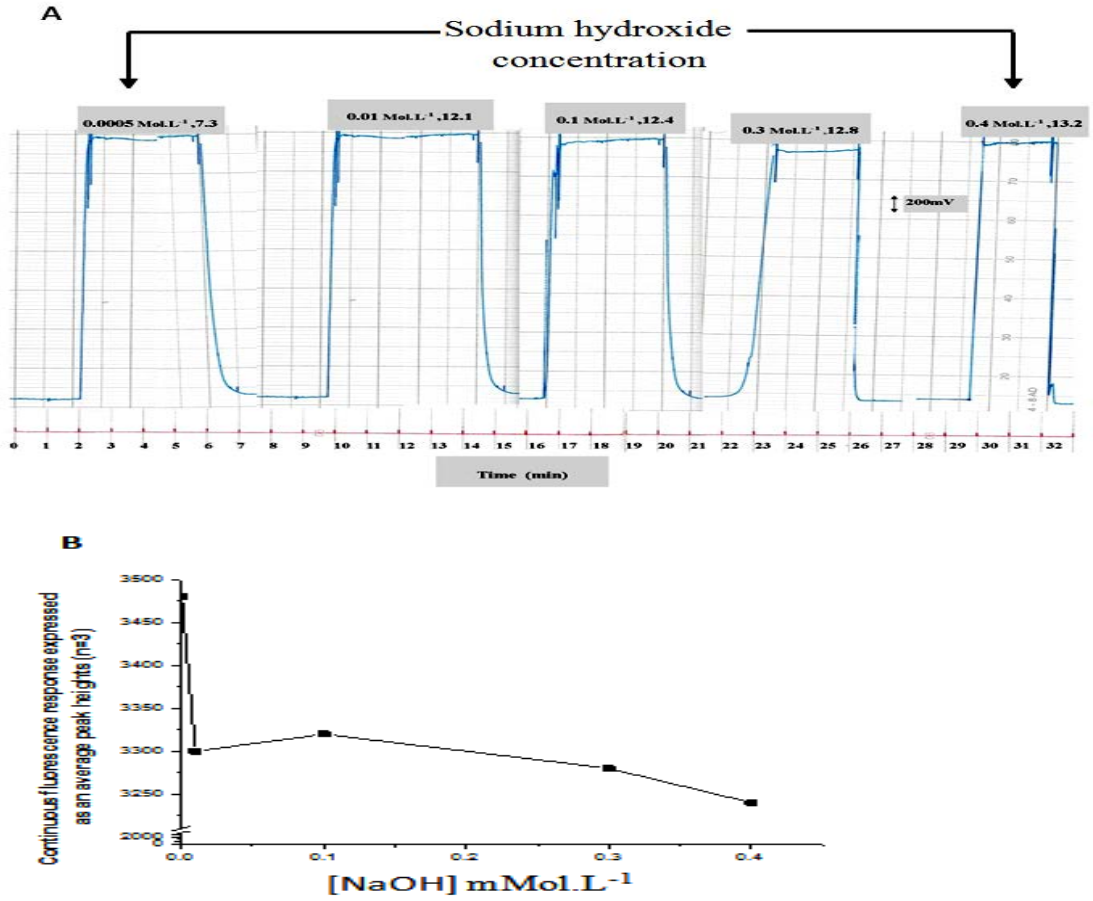


Figure 3-A-Variation of sodium hydroxide concentration on response profile
B-Variation of sodium hydroxide on continuous fluorescence response

Table 2-Variation of sodium hydroxide concentration effect on continuous fluorescence response using close valve mode

pH	Concentration of sodium hydroxide Mol.L^{-1}	Continuous of fluorescence response expressed as an average peak heights (n=3) $\bar{y}_i$ in mV	RSD%	Confidence interval of the average response (95% confidence level) $\bar{y}_i \pm t_{0.05/2,\ n-1}\ \sigma_{n-1}/\sqrt{n}$
7.3	0.0005	3480	0	3480±0
12.1	0.01	3300	0.03	3300±2.48
12.4	0.1	3320	0.05	3320±3.78
12.8	0.3	3280	0.13	3280±10.33
13.2	0.4	3240	0.06	3240±4.97

Effect of iodide ion for I^-- IO_3^- -H_3O^+ system

The effect of iodide ion on the sensitivity of quenching of fluorescence was studied. A series of solutions (0.01-5 mMol.L^{-1}) at fixed concentration of iodate ion (0.1 mMol.L^{-1}) which injected at valve no.$_2$ (35µL) while valve no.$_1$ injected oxonium ion (H_2SO_4 0.1 mMol.L^{-1}) at flow rate 1.3mL/min for fluorescien solution (0.5 mMol.L^{-1}) using sequential open valve mode . The obtained results shown in figure-4.A,B and table 3. It was noticed that an increase in the I^- leads to an increase in the iodine released from I^-- IO_3^- -H_3O^+system , which in turn affect on the quenching of fluorescence for fluorescien solution up to 1 mMol.L^{-1}, while at higher concentration(more than 1

mMol.L^{-1}) a slightly increase of the quenching fluorescence response therefore 1 mMol.L^{-1} was chosen as the optimum concentration that used in all subsequent experiments

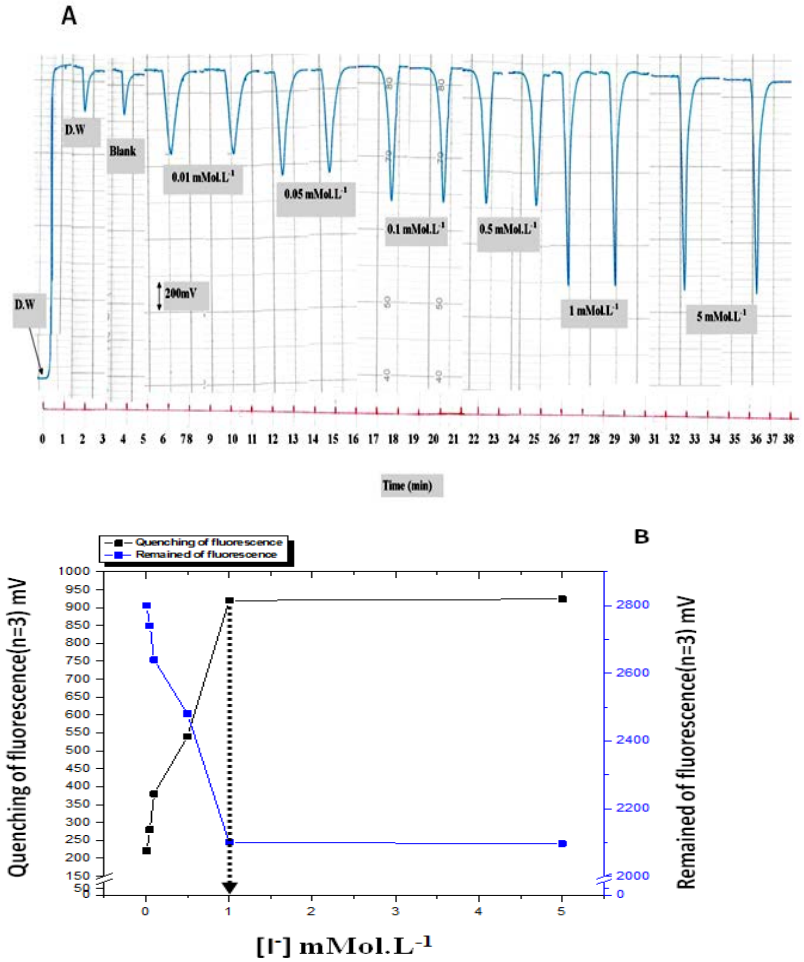


Figure 4-A-Effect of variation of iodide ion concentration on the response time profile
B-Variation of iodide ion concentration on quenching of fluorescence and remained of fluorescence

Table 3-Variation of iodide ion concentration on total quenching of fluorescence expressed as an average peak heights (n=3)

Concentration of I^- mMol.L^{-1}	**Total quenching of fluorescence expressed as an average peak heights (n=3) $\bar{y}_i$ in mV**	**Quenching of fluorescence $\bar{y}_{Qi}$ (n=3)mV**	**RSD%**	**Confidence interval of the average response (95% confidence level) $\bar{y}_i \pm t_{0.05/2,\, n-1}\ \sigma_{n-1}/\sqrt{n}$**	**Remained of fluorescence $\bar{y}_{Ri}$ (n=3)mV**
0.01	680	220	0.29	680±4.97	2800
0.05	740	280	0.47	740±8.59	2740
0.1	840	380	0.24	840±4.97	2640
0.5	1000	540	0.10	1000±2.48	2480
1	1360	900	0.29	1360±9.94	2120
5	1364	904	0.07	1364±2.48	2116

Response of continuous fluorescence : 3480mV, Response of blank : 460mV

Variation of iodate ion concentration at fixed iodide ion concentration

A series of IO_3^- solutions (0.01-5 mMol.L^{-1}) at fixed concentration of iodide ion (1 mMol.L^{-1}) were prepared, while maintaining other variables as in previous experiments are constant. Each measurement was repeated three time successively & using sequential open valve mode. Figure-5 A,B shows various response profile versus variation of iodate ion concentration and tabulated the data in

table-4. The results show the effect of the increase of IO_3^- concentration on the sensitivity for quenching of fluorescence to generate the iodine leading to might be lose energy of excited fluorescent molecules via absorption of energy by I_2 or any other molecules with the fluorescent molecule medium. At more than 0.1 mMol.L^{-1} a constant of response followed by slightly decreased in response expressed as a quenching of fluorescence. Therefore 0.1 mMol.L^{-1} was regarded as the optimum concentration.

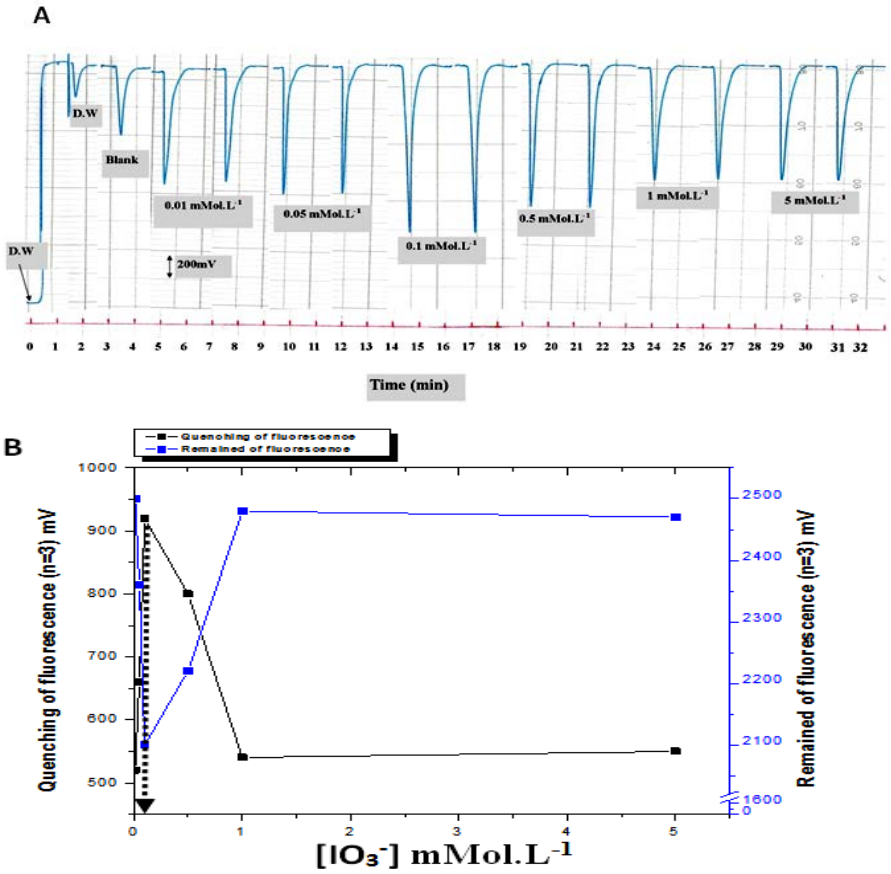


Figure 5-Effect of variation of iodate ion on the:A-Response profile B- Quenching and remained of fluorescence

Table 4-Effect of iodate ion on the quenching of fluorescence by I_2 for I^-- IO_3^- -H_3O^+ system

Concentration of IO_3^- mMol.L^{-1}	**Total quenching of fluorescence expressed as an average peak heights(n=3)$\bar{y}_i$ in mV**	**Quenching of fluorescence mV $\bar{y}_{Qi}$ (n=3)mV**	**RSD%**	**Confidence interval of the average response (95% confidence level) $\bar{y}_i \pm t_{0.05/2,\, n-1}\ \sigma_{n-1}/\sqrt{n}$**	**Remained of fluorescence $\bar{y}_{Ri}$ (n=3)mV**
0.01	980	520	0.23	980±5.71	2500
0.05	1120	660	0.22	1120±6.24	2360
0.1	1380	920	0.29	1380±9.84	2100
0.5	1260	800	0.16	1260±4.97	2220
1	1000	540	0.4	1000±9.94	2480
5	1010	550	0.19	1010±4.97	2470

Response of continuous fluorescence : 3480mV, Response of blank : 460mV

Physical parameter

Flow rate

The effect of the flow rate of fluorescien line(0.5 mMol.L^{-1}) as a carrier stream on the continuous fluorescence signal was investigated by changing their flow rate from 0.2 to 1.75mL/min . Using 35µL as the injected sample volume of both valve no.$_1$ (0.1 mMol.L^{-1} sulfuric acid) and valve no.$_2$ (1 mMol.L^{-1} I^- -0.1 mMol.L^{-1} IO_3^-) using sequential open valve mode . The obtained response profile

shown in figure-6.A and the results tabulated in table -5. It was noticed that at low flow rate there was an increase in dilution and dispersion which might cause an increase in peak width (Δt_b) as shown in figure-6.B while at higher flow rate(> 0.8mL/min), a regular response and very sharp maxima was obtained . 1.3 mL/min was chosen as an optimum flow rate as a clear peak profile response , short analysis time , narrow Δt_b and easy measurable peak .

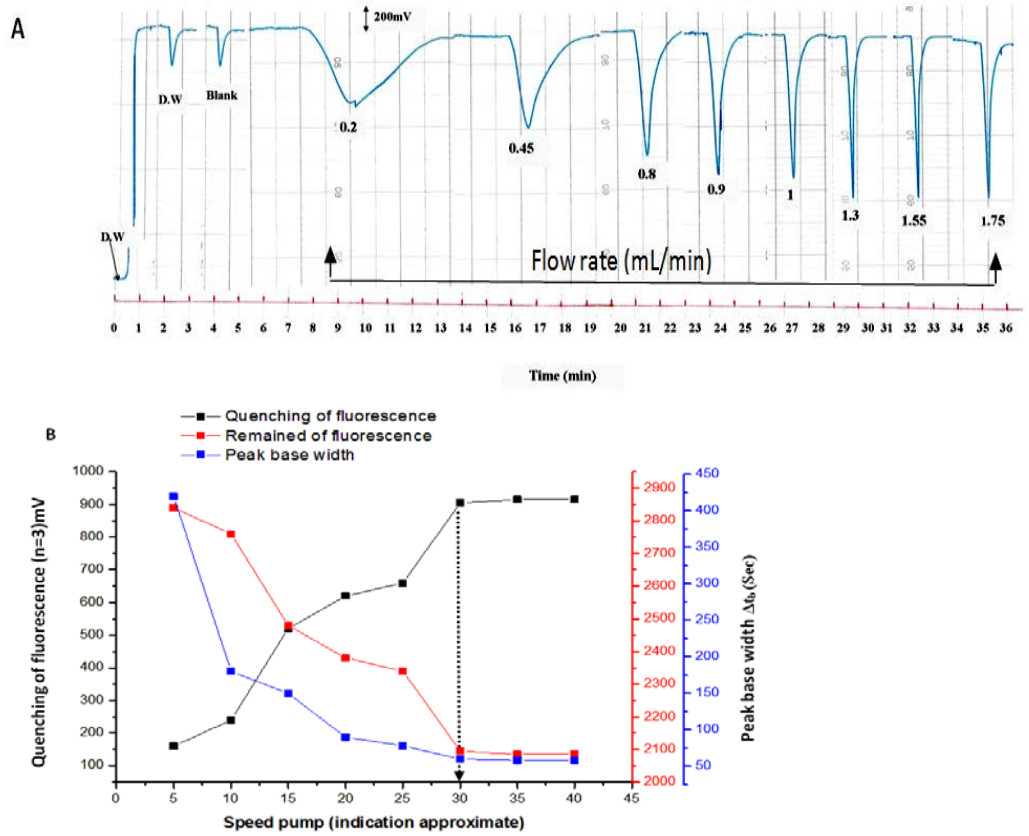


Figure 6- A-Effect of variation of flow rate on quenching of fluorescence using I^- - IO_3^-- H_3O^+ system to generate I_2 .B- Variation of flow rate on :quenching of fluorescence, remained of fluorescence and peak base width Δt_b (Sec)

Table 5: Variation effect of flow rate on quenching of fluorescence response

Speed of peristaltic pump (indication approximate)	Flow rate(mL/ min)	Total quenching of fluorescence expressed as an average peak heights(n=3) $\bar{y}_i$ in mV	Quenching of fluorescence $\bar{y}_{Qi}$ (n=3)mV	RSD%	Confidence interval of the average response (95% confidence) $\bar{y}_i \pm t_{0.05/2,\, n-1} \ \sigma_{n-1}/\sqrt{n}$	Remained of fluorescence $\bar{y}_{Ri}$ (n=3)mV	Δt_b (Sec)
5	0.2	620	160	0.65	620±9.94	2840	420
10	0.45	700	240	0.04	700±0.77	2760	180
15	0.8	980	520	0.2	980±4.97	2480	150
20	0.9	1080	620	0.09	1080±2.48	2380	90
25	1	1120	660	0.18	1120±4.97	2340	78
30	1.3	1365	905	0.01	1365±0.49	2095	60
35	1.55	1375	915	0.05	1375±1.74	2085	60
40	1.75	1375	915	0	1375±0	2085	60

Response of continuous fluorescence : 3460mV, Response of blank : 460mV
Δt_b (sec) : Time lapse for the fluorescence response within measuring cell or peak base width

Effect of sample volume

Sample volume no.$_2$

Using the optimum parameters achieved in previous sections. The effect of sample volume no.$_2$ using selected sample volume of loop no.$_1$ (35µL) with 0.1 mMol.L^{-1} sulfuric acid as an analyte was used. Variable sample volume (18-43µL) were injected using sequential open valve mode. The obtained results are shown in figure-7.A,B and the data tabulated in table 6.It was noticed that an increase in sample volume via second valve which supply the compliment chemicals (i.e.I^- &IO_3^-) to generate I_2 which will cause the increase of response expressed as a quenching of fluorescence reaching to 39µL. More than39µL, it was noticed a decrease in response height which could be attributed to the an increase of I^- &IO_3^- segment, but does not satisfy enough to the available concentration of acid (H_3O^+). Therefore and based on this postulate, I_2 is not enough to an increase the quenching effect, so; 39µL was chosen as an optimum sample volume no.$_2$ segment.

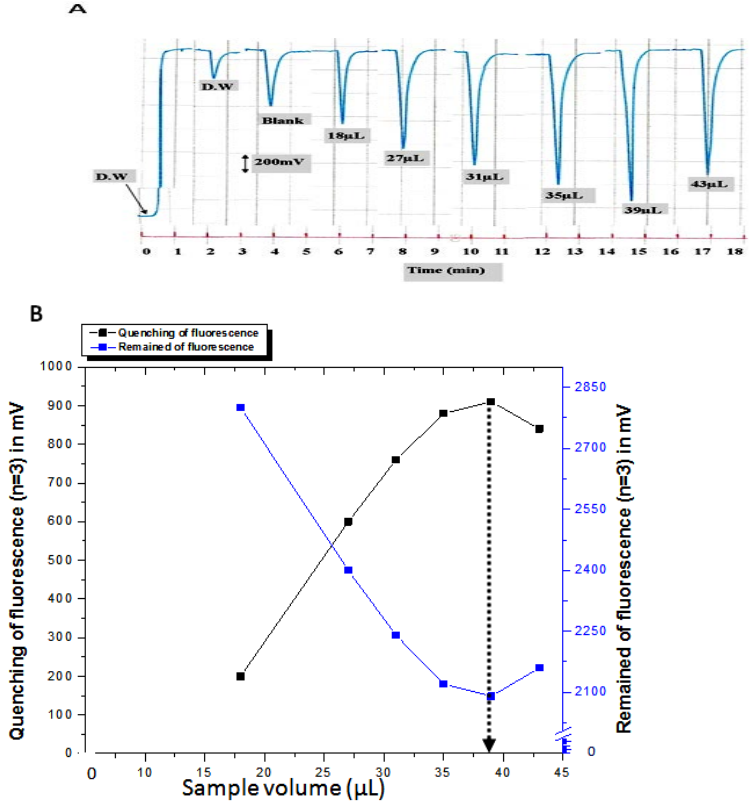


Figure 7- Variation of sample volume segment at valve no.$_2$ on: A-response time profile B-Quenching of fluorescence and remained of fluorescence

Table 6-Variation of injected sample volume at valve no.$_2$ using I^- -IO_3^--H_3O^+system

Length of sample loop(cm) Diameter (0.5mm)	Sample volume (µL)	Total quenching of fluorescence expressed as an average peak heights (n=3) $\bar{y}_i$ in mV	Quenching of fluorescence $\bar{y}_{Oi}$ (n=3)mV	RSD%	Confidence interval of the average response (95% confidence level) $\bar{y}_i \pm t_{0.05/2,\, n-1}\ \sigma_{n-1}/\sqrt{n}$	Remained of fluorescence $\bar{y}_{Ri}$ (n=3)mV	Δt_b(Sec.)
9	18	660	200	0.26	660±4.26	2800	48
14	27	1060	600	0.09	1060±2.37	2400	60
16	31	1220	760	0.04	1220±1.21	2240	60
18	35	1340	880	0.01	1340±0.33	2120	66
20	39	1370	910	0.02	1370±0.68	2090	72
22	43	1300	840	0.04	1300±1.29	2160	78

Δt_b (Sec): Time lapse for the fluorescence response within measuring cell, Response of continuous fluorescence :3460mV , Response of blank : 460mV

Sample volume no.$_1$

Sample volume no.$_1$ was carried out using 39µL as the optimum of sample volume no.$_2$ while the other parameters are fixed. A set of different sample loop volumes of valve no.$_1$ ranging from 18-45µL; using sequential open valves mode was studied.Fig.8 shows that an increase in sensitivity for quenching of fluorescence with increase in sample volume of H_3O^+ at valve no.$_1$ up to 35µL. Above 35µL there was a slightly decrease in quenching effect, which most probably attributed to the consumption some of the sodium hydroxide by acid these in turn to lead decrease of continuous fluorescence . The optimum sample volume 35µL gave regular and highest responses of quenching fluorescence depending on I^- -IO_3^--H_3O^+ system. The obtained results are tabulated in table- 7.

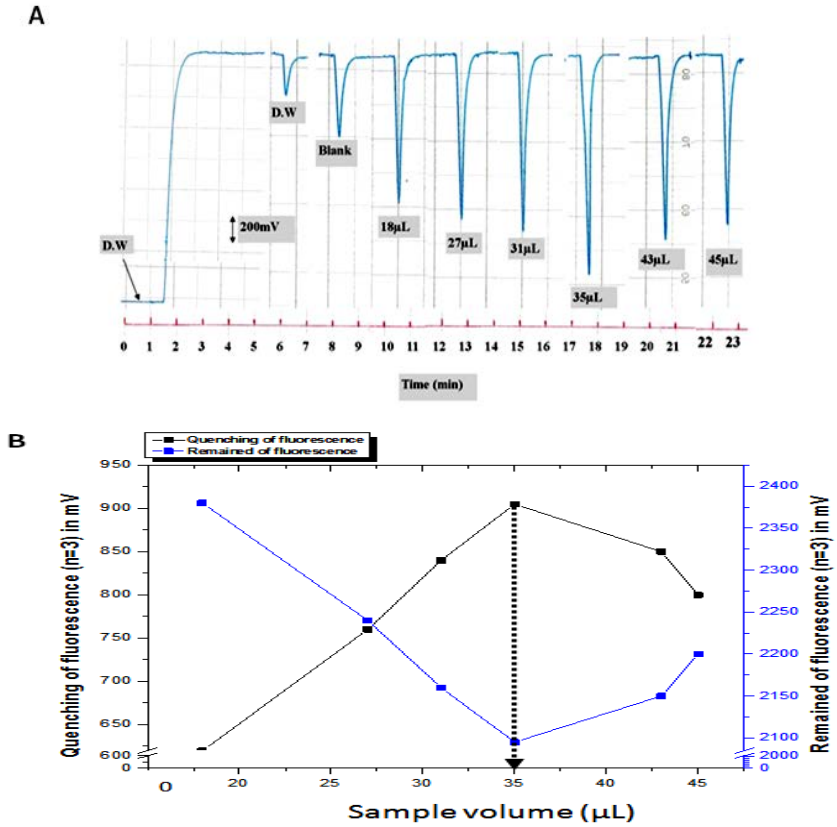


Figure 8-Effect of sample volume at valve no.$_1$ on: A- response profile - time
B- Quenching of fluorescence and remained of fluorescence

Table 7: Effect of sample volume at valve no.$_1$ on the measurement of fluorescence response

Length of sample loop(cm) (D=0.5mm)	Sample volume $=r^2\pi h$ (µL)	Total quenching of fluorescence expressed as an average peak heights (n=3) $\bar{y}_i$ in mV	Quenching of fluorescence $\bar{y}_{Oi}$ (n=3)mV	RSD%	Confidence interval of the average response (95% confidence level) $\bar{y}_i \pm t_{0.05/2,\, n-1}\ \sigma_{n-1}/\sqrt{n}$	Remained of fluorescence $\bar{y}_{Ri}$ (n=3)mV	Δt_b (Sec.)
9	18	1080	620	0.06	1080±1.61	2380	60
14	27	1220	760	0.04	1220±1.21	2240	66
16	31	1300	840	0.05	1300±1.61	2160	72
18	35	1365	905	0.02	1365±0.68	2095	72
22	43	1310	850	0.07	1310±2.28	2150	78
23.1	45	1260	800	0.02	1260±0.63	2200	84

Δt_b (Sec): Time lapse for the fluorescence response within measuring cell or peak base width
Response of continuous fluorescence : 3460mV, Response of blank : 460mV

Purge time

Using optimum parameters that were achieved in the previous sections, purge time of the sample volume no.$_1$ and sample volume no.$_2$ to be injected via the fluorescien stream was studied. Using different purge time (2-32 sec) for the sample segment no.$_1$ (oxonuim ion) and sample segment no.$_2$ (I^- -IO_3^-) to pass through both injection valves successively at the same time at pre-selected time interval as shown in table 8 column no.$_1$.Also open valves & sequential pattern open valve mode was used i.e.sample segment in loop no.$_1$ (i.e.valve no.$_1$) is sent followed by a delay of 2 seconds then sample no.$_2$ (i.e. valve no.$_2$) was introduced .It was noticed (figure-9.A,B) that opening both injection valves at the same time and different times causes a disturbed deformed profile of responses and a broadening in Δt_b(base width), this might be due to unsynchronized meeting and merging zones of both ingredient available at the two valves. While taking the synchronize effect i.e. giving enough time for reaction between I^- -IO_3^- with H_3O^+ and merging will induce sharp peaks and smooth responses . Therefore sequential open valve was chosen for the next studies. All results were tabulated in table - 8.

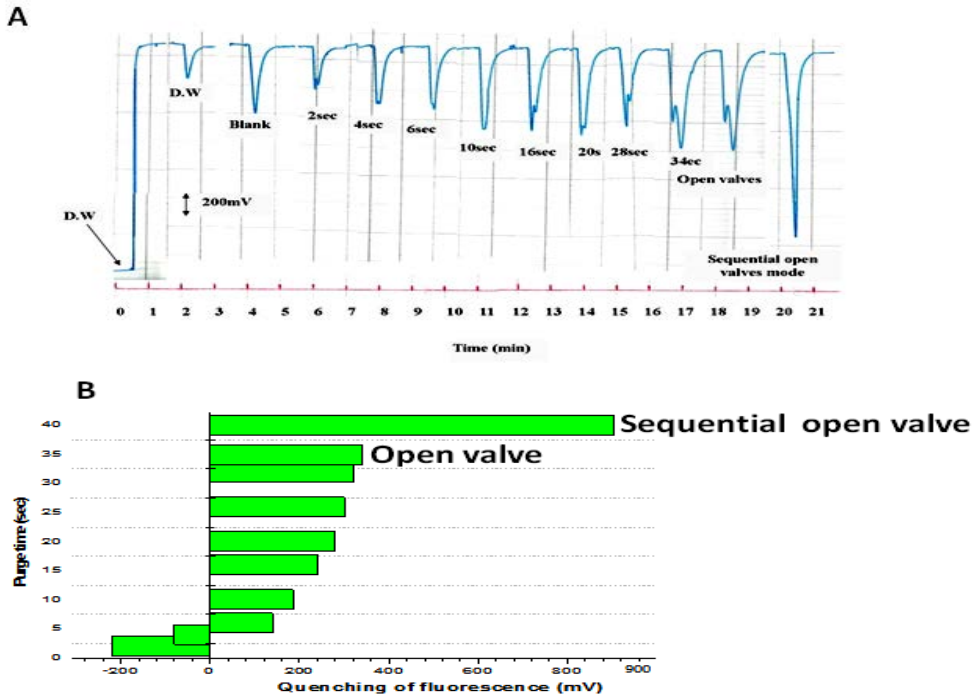


Figure 9- Effect of purge time on : A-response time profile B-quenching of fluorescence

Table 8- Variation of purge time on the total quenching of fluorescence response using I^- -IO_3^--H_3O^+ system

Purge time(sec)	Total quenching of fluorescence expressed as an average peak heights (n=3) $\bar{y}_i$ in mV	Quenching of fluorescence $\bar{y}_{Qi}$ (n=3)mV	RSD%	Confidence interval of the average response (95% confidence level)$\bar{y}_i \pm t_{0.05/2,\ n-1}\ \sigma_{n-1}/\sqrt{n}$	Remained of fluorescence $\bar{y}_{Ri}$ (n=3)mV
2	240	-220	0.83	240±4.97	3220
4	380	-80	0.61	380±5.71	3080
6	600	140	0.16	600±2.48	2860
10	645	185	0.62	645±9.94	2815
16	700	240	0.14	700±2.48	2760
20	740	280	0.1	740±1.86	2720
26	760	300	0.03	760±0.57	2700
32	780	320	0.29	780±5.71	2680
Open valves	800	340	0.19	800±3.73	2660
Sequential	1365	905	0.02	1365±0.68	2095

open valves					

Response of continuous fluorescence : 3460mV, Response of blank : 460mV

Effect of reaction coil length

The effect of reaction coil on response profile was studied. Using variable coil length 0-100 cm which connected after injection valve no.$_2$ directly (figure-1A) then passing through the flow cell . Using optimum concentration of fluorescien solution (0.5 mMol.L^{-1}) and selected concentration of sulphuric acid 0.1 mMol.L^{-1} (35µL) at optimum flow rate 1.3mL/min were used. Figure-10.A,B shows the effect of coil length on quenching of fluorescence response . It can be seen that a decrease in quenching effect with increase of coil length, which might probably attributed to the increase effect of dilution and dispersion on I_2 segment released from I^- -IO_3^--H_3O^+ system, these in turn to lead a decrease in quench of fluorescence. All this study indicate that no delay coil is necessary for the determination of acid by quenching of fluorescence with one line manifold design system .All results were tabulated in table -9

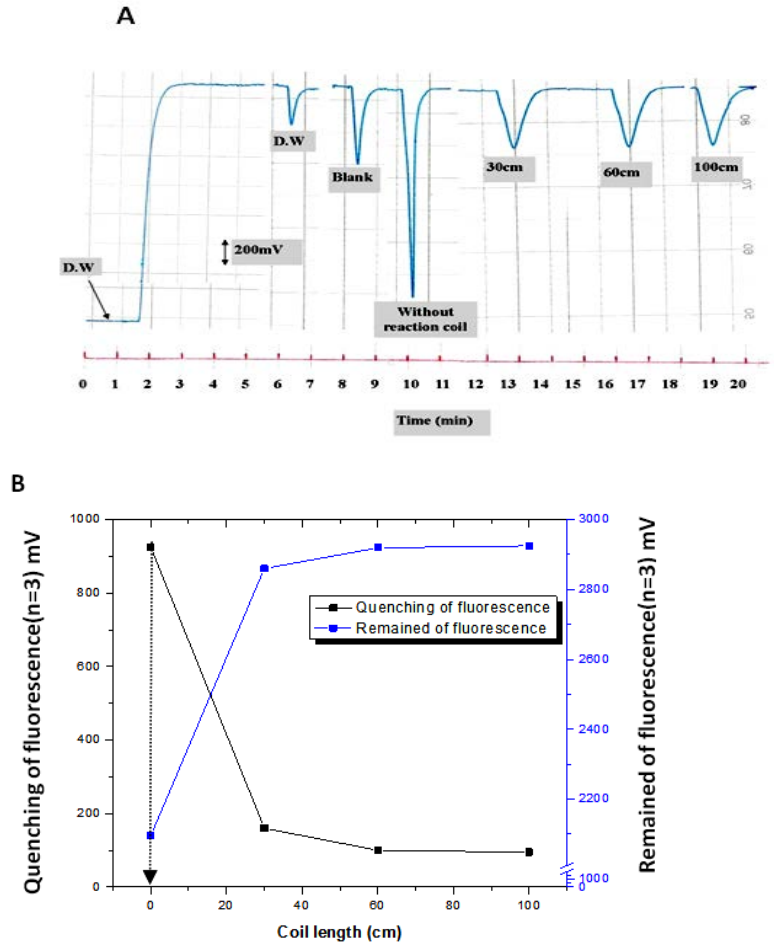


Figure 10- Variation of coil length on; A- Response time profile using I^- -IO_3^--H_3O^+ quenching system , B- quenching and remained of fluorescence

Table 9: Variation of coil length on total quenching of fluorescence response expressed as an average peak heights (n=3)

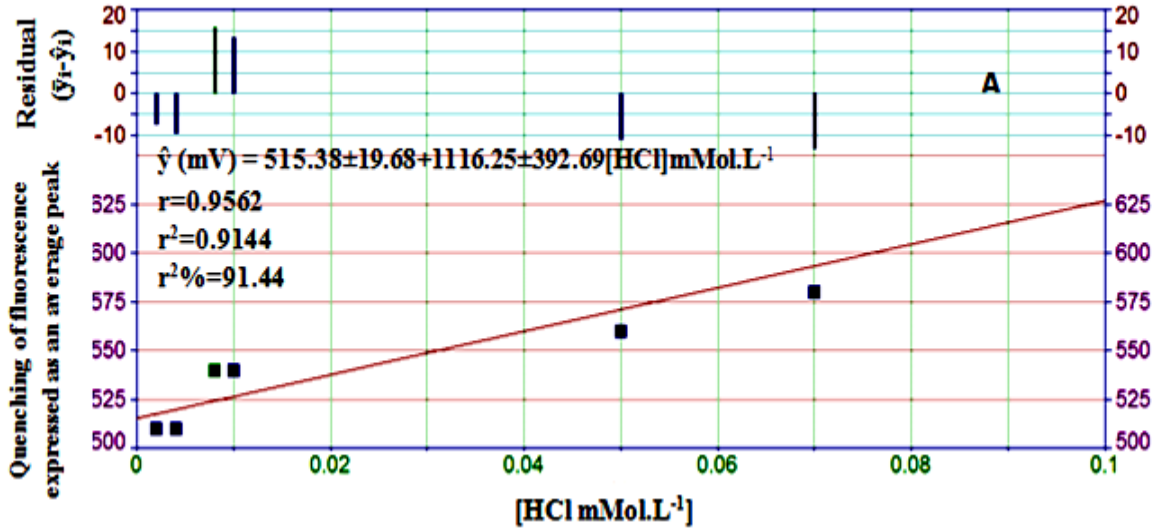
Coil length (cm)	Coil volume(mL) $r^2\pi h$	Total quenching of fluorescence expressed as an average peak heights (n=3) $\bar{y}_i$ in mV	Quenching of fluorescence $\bar{y}_{Qi}$ (n=3)mV	RSD%	Confidence interval of the average response (95% confidence level) $\bar{y}_i \pm t_{0.05/2,\, n-1}\ \sigma_{n-1}/\sqrt{n}$	Remained of fluorescence $\bar{y}_{Ri}$ (n=3)mV
0	0	1364	924	0.07	1364±2.48	2096
30	0.235	600	160	0.04	600±0.62	2860
60	0.471	540	100	0.27	540±3.73	2920

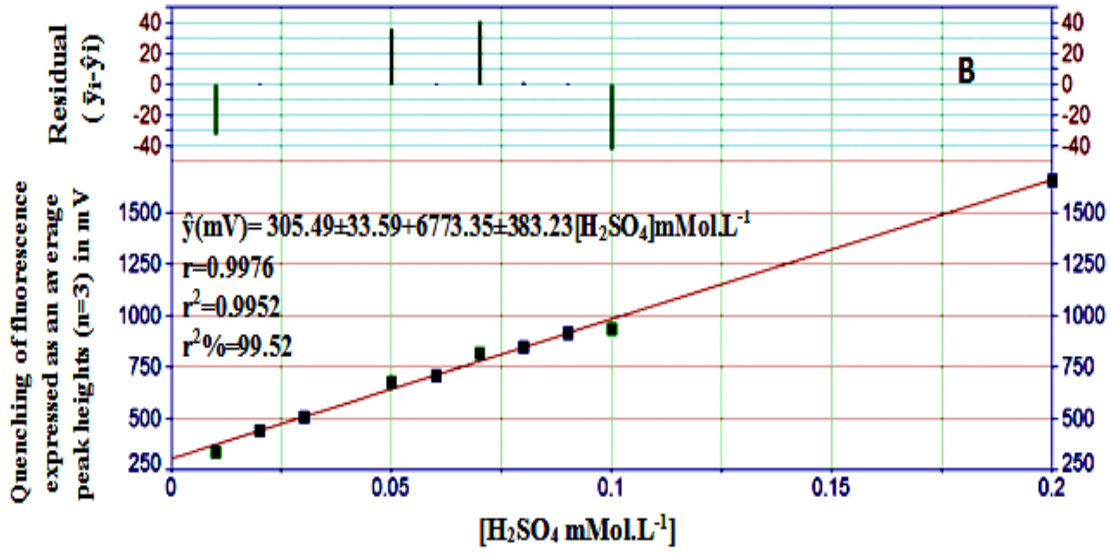
100	0.785	535	95	0.06	535±0.75	2925

Response of continuous fluorescence : 3460mV, Response of blank : 440mV

Calibration curve

A series of acid solution having the concentrations ranging from 0.002-3 mMol.L^{-1} using 35µL as an injected sample volume with all optimum parameters achieved in previous sections for each acid (HCl, H_2SO_4, $HClO_4$ and tartaric acid) . Total quenching of fluorescence , quenching of fluorescence and remained of fluorescence (mV) was plotted against the concentration of acid. Table 10 tabulated all the results obtained. It can be noticed that the increase in acid concentration whatever its sources leads to a deviation from straight line and decreases the numerical value of correlation coefficient. This might be probably attributed that an increased concentration of acid i.e. unconsumed by I^--IO_3^--H_3O^+system might start to react with the alkali (NaOH solution) that is used for dissolution of fluorescien (free acid). Fig.11 shows that the range of calibration graph for each acid at 95% confidence level[21].





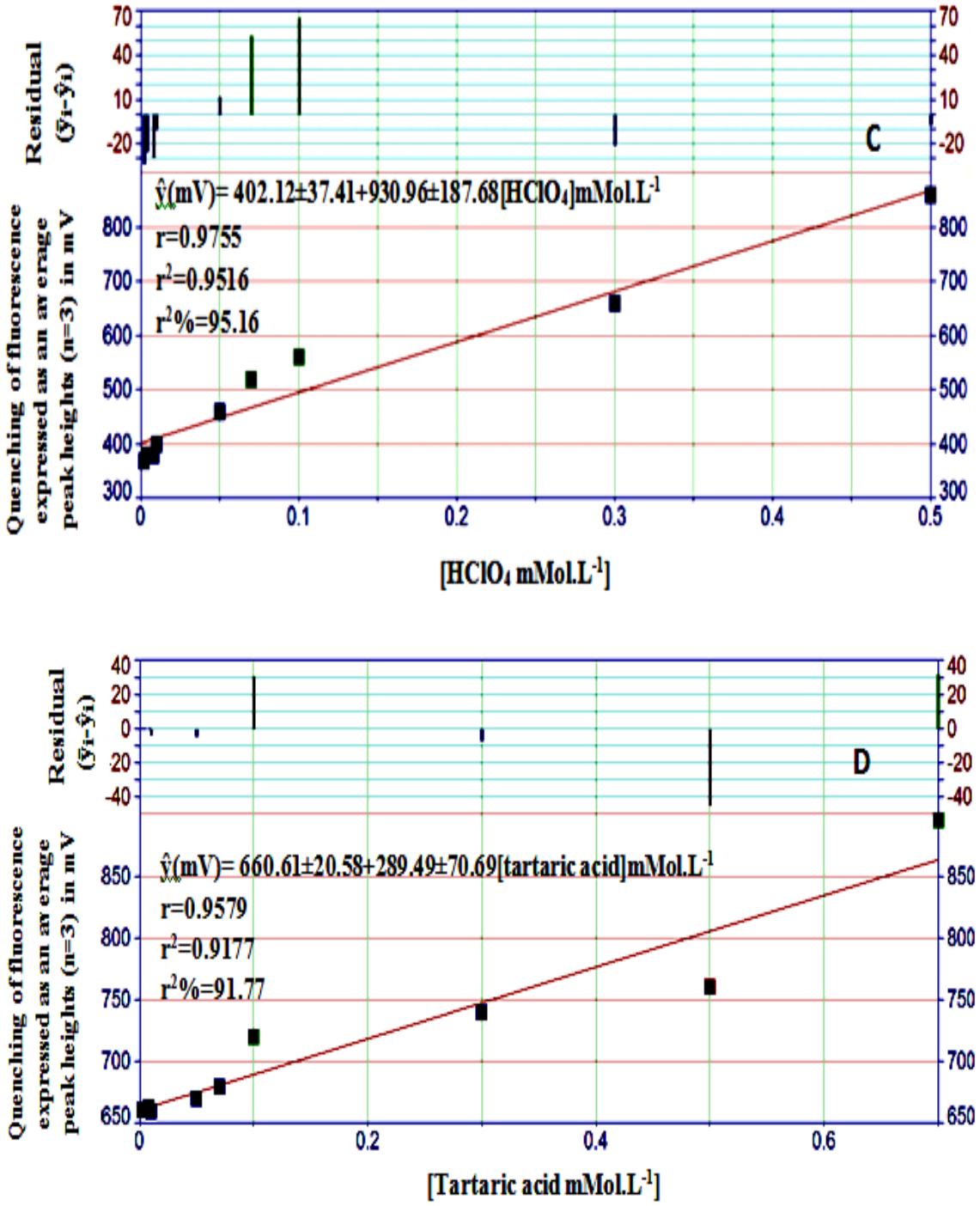


Figure 11-Calibration gragh for variaton of $[H_3O^+]$ mMol.L^{-1} on quenching of fluorescence($\hat{y}_i$) using I^- - IO_3^--H_3O^+ system: A-HCl , B-H_2SO_4 , C-$HClO_4$ and D-Tartaric acid. $\bar{y}_i$=practical value $\hat{y}_i$=estimated value

Table 10-Summary of linear regression equation for the variation of total quenching of fluorescence, quenching of fluorescence and remained of fluorescence response with variation of acids concentration at optimum condition

Type of acid	**Measured $[H_3O^+]$ mMol.L^{-1}**	**Linear dynamic range mMol.L^{-1}**	**Type of measurement**	**$\hat{y}$(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	0.002-3	n=7 0.002-0.1	Total quenching of fluorescence	955.38±19.69+1116.25±392.69[HCl]mMol.L^{-1}	0.9562 0.9144 91.44	2.57<< 7.31	
			Quenching of fluorescence	515.38±19.68+1116.25±392.69[HCl] mMol.L^{-1}			
			Remained of fluorescence	2504.62±19.69-1116.25±392.69[HCl] mMol.L^{-1}			
H_2SO_4 Ka=10^3	0.002-3	n=10 0.01-0.2	Total quenching of fluorescence	745.49±33.59+6773.35±383.23[H_2SO_4] mMol.L^{-1}	0.9976 0.9952 99.52	2.31<< 40.75	
			Quenching of fluorescence	305.49±33.59+6773.35±383.23[H_2SO_4] mMol.L^{-1}			
			Remained of fluorescence	2714.51±33.59-6773.35±383.23[H_2SO_4] mMol.L^{-1}			
$HClO_4$ Ka=10^3	0.002-3	n=9 0.002-0.5	Total quenching of fluorescence	842.12±37.41+930.96±187.68[$HClO_4$] mMol.L^{-1}	0.9755 0.9516 95.16	2.37<< 11.73	
			Quenching of fluorescence	402.12±37.41+930.96±187.68[$HClO_4$] mMol.L^{-1}			
			Remained of fluorescence	2617.88±37.41-930.96±187.68[$HclO_4$] mMol.L^{-1}			
Tartaric acid Ka_1= 1.29x10^{-3} Ka_2= 3.98x10^{-5}	0.002-3	n=10 0.002-0.7	Total quenching of fluorescence	1100.61±20.58+289.49±70.69[tartaric acid] mMol.L^{-1}	0.9579 0.9177 91.77	2.31<< 9.44	
			Quenching of fluorescence	660.61±20.58+289.49±70.69[tartaric acid] mMol.L^{-1}			
			Remained of fluorescence	2359.39±20.58-289.49±70.69[tartaric acid] mMol.L^{-1}			

$\hat{y}$: 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.

A comparison study was carried out between different acids concerning their sensitivity to the adopted used method (table no.10). It was noticed that the ratio of slopes of the linear regression plot for sulphuric acid to HCl acid =(6773.35/1116.25) = 6.068.This indicate that a six fold sensitivity is obtained. The same mode of treatment was applied to estimate the slope ratio of perchloric acid /

tartaric acid which is equal to: 930.96/289.49=3.22 which indicate that more than three fold sensitivity can be achieved for $HClO_4$ acid relative to tartaric acid.

Limit of detection

The limit of detection of analyte (oxonium ion) was calculated using two different approach as tabulated in table 11for all acids as an injected sample volume of 35µL.

Table 11-Detection limit of all acids using I^- -IO_3^- -H_3O^+ system

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	0.001	1.28ng/sample	1.71 ng/sample
H_2SO_4	0.005	17.16ng/sample	0.76ng/sample
$HClO_4$	0.001	3.52ng/sample	5.66ng/sample
Tartaric acid	0.001	5.25ng/sample	27.22ng/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 reality and repeatability of the proposed method was studied at a selected concentration of all acids (0.01 $mMol.L^{-1}$). A repeat measurements for eight successive injection were measured and the obtained results are tabulated in table -12 while figure-12 shows a kind of response profile for all acids.

Table 12-Repeatability of HCl, H_2SO_4, $HClO_4$ and tartaric acid results obtained for the quenching of fluorescence system

Type of acid 0.01$mMol.L^{-1}$	**No. of injection**	**Average of total quenching of fluorescence expressed as an average peak heights $\bar{y}_i$ in mV**	**Quenching of fluorescence $\bar{y}_{Qi}$ (n=3)mV**	**RSD%**	**Confidence interval of the average response (95% confidence level) $\bar{y}_i \pm t_{0.05/2,\, n-1}\ \sigma_{n-1}/\sqrt{n}$**
HCl	**8**	**980**	**540**	**0.12**	**980±0.98**
H_2SO_4	**8**	**780**	**340**	**0.45**	**780±2.93**
$HClO_4$	**8**	**841**	**401**	**0.18**	**841±1.27**
Tartaric acid	**8**	**1100**	**660**	**0.18**	**1100±1.66**

Response of continuous fluorescence=3460mV , Response of blank : 440mV, $t_{0.05/2,\ 7=2.365}$

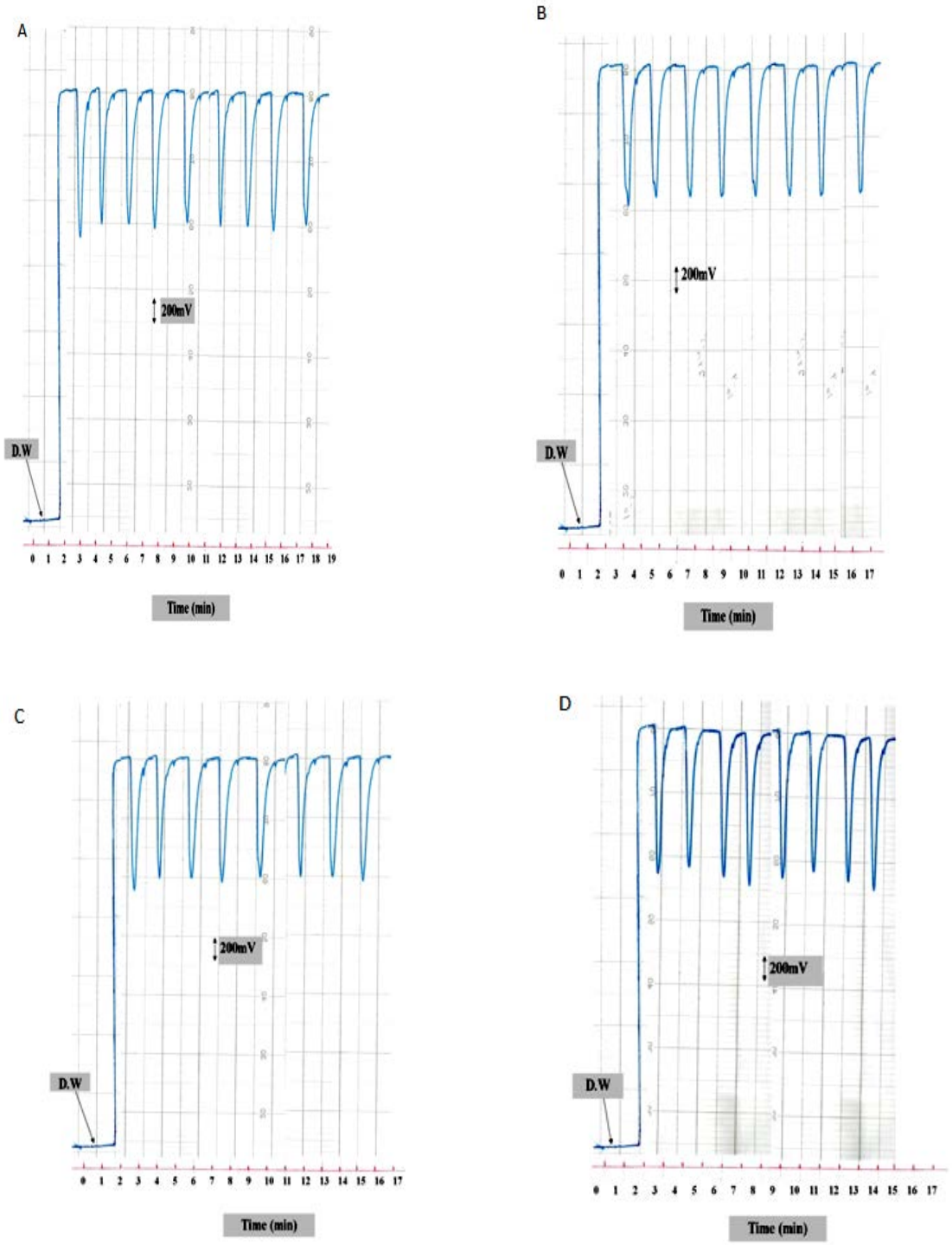


Figure 12 -Response profile of successive repeatability measurements of : A-HCl ,B-H_2SO_4, C- $HClO_4$ and D-Tartaric acid using quenching of fluorescence by I^- -IO_3^- -H_3O^+ system

Classical method

Calibration graph of classical method using calibrated pH meter was made to determination of oxonium ion in the range (0.01-1.5 mMol.L^{-1}) for used acid. Table -13 tabulates all obtained data for all acids depending on the classical method measurements.

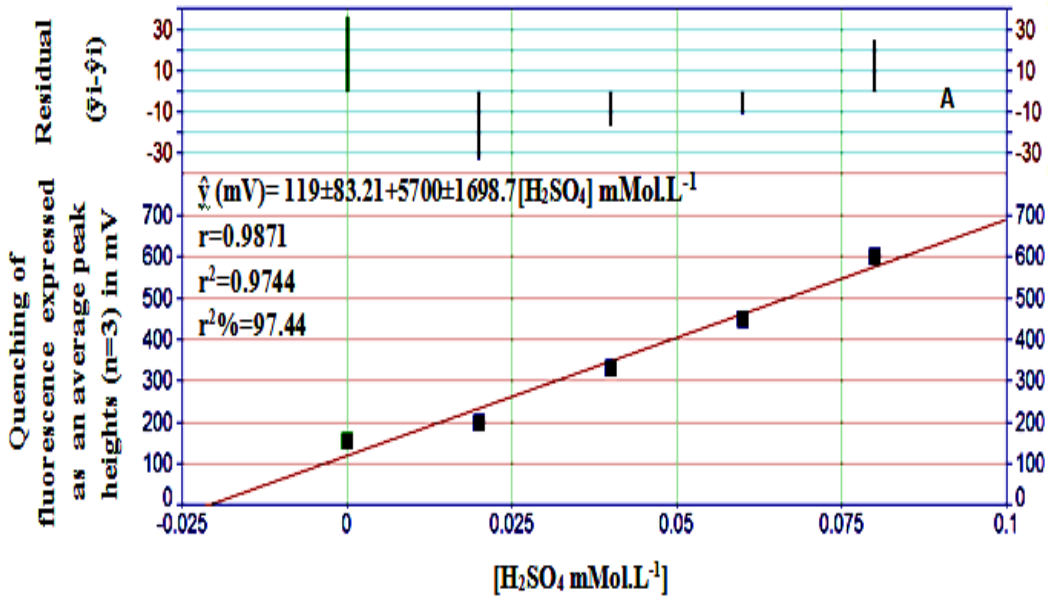
Table 13-Summary of calibration graph results for the determination of HCl, H_2SO_4, $HClO_4$ and tartaric acid using pH-meter

Type of acid	Measured $[H_3O^+]$ $mMol.L^{-1}$ n=9	$\hat{y}(mV)=(a\pm S_a t)+(b\pm S_b t)[H_3O^+]mMol.L^{-1}$ at confidence level 95%, n-2	r r^2 r^2%	t_{tab} at 95% confidence level, n-2	$t_{cal}=\frac{\lvert r\rvert\sqrt{n-2}}{\sqrt{1-r^2}}$
HCl	0.01-1.5	23.56±7.71+82.26±11.45[HCl]$mMol.L^{-1}$	0.9881 0.9763 97.63	2.365<< 17	
H_2SO_4	0.01-1.5	73.57±4.54+37.55±6.72[H_2SO_4] $mMol.L^{-1}$	0.9806 0.9615 96.15	2.365<< 13.22	
$HClO_4$	0.01-1.5	53.23±8.37+62.86±12.39[$HClO_4$] $mMol.L^{-1}$	0.9766 0.9535 95.35	2.365<< 11.99	
Tartaric acid	0.01-1.3	15.26±8.39+78.07±13.41[Tartaric acid] $mMol.L^{-1}$	0.9821 0.9644 96.44	2.365<< 13.78	

$\hat{y}$: estimated response (mV) for (n=3) expressed as 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. $t_{0.05/2,\ 7=2.365}$

Application

The proposed method (laser diode fluorimeter) was used for the determination of oxonium ion in two samples (H_2SO_4,98%,Loba Chemie –India&tartaric acid ,Thomas Baker-India). A series of solution were prepared of each sample (50 $mMol.L^{-1}$) by transferring 0.01mL to each of the five volumetric flask (25mL), followed by the addition of 0.0, 0.01, 0.02, 0.03 and 0.04mL from 50$mMol.L^{-1}$ of standard solution of acid in order to have the concentration range of 0.0-0.08 $mMol.L^{-1}$ to constructed the standard addition calibration curve(figure-13 A,B). The measurements were conducted by both methods (Laser diode fluorimeter&pH-meter). The results were tabulated in table 14.A at confidence interval 95%.



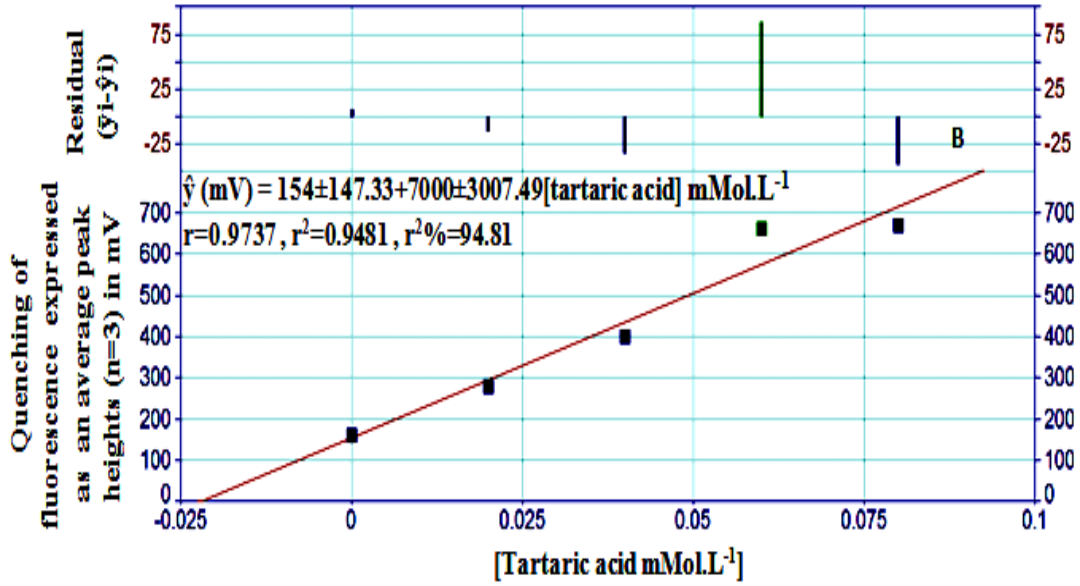


Figure 13: Standard addition calibration graph using laser diode fluorimeter for : A- H_2SO_4 B- Tartaric acid. Residual = (ȳi-ŷi) in mV, ȳi = practical value, ŷi =estimated value.

Table 14-A: 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.

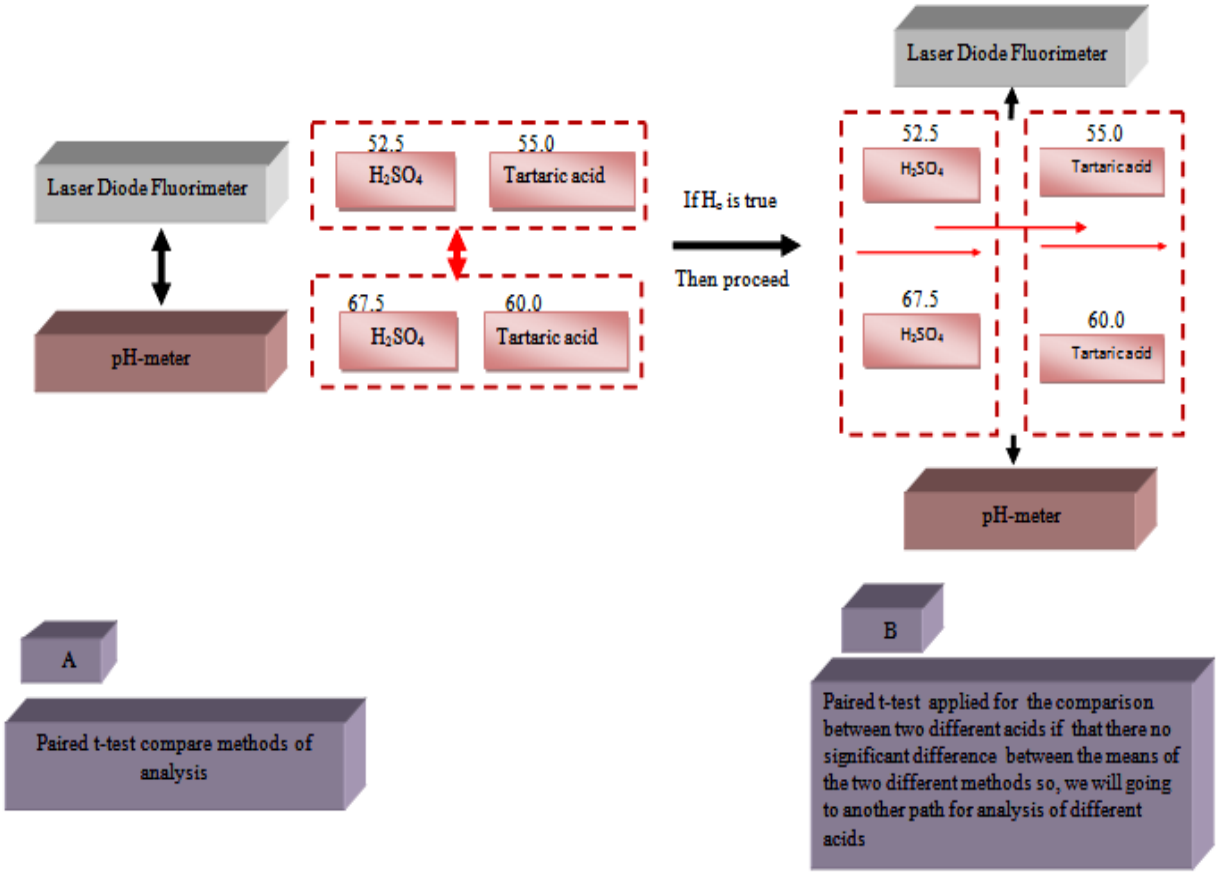
No. of Sample	Company and country of sample	Laser Diode Fluorimeter / pH-meter					ŷ=a± S_at+ b± S_bt[H_3O^+]mMol.L^{-1} at confidence level 95%, n-2	r / r^2 / r^2%	Practical concentration mMol.L^{-1} in 25mL / In prepared sample (50mMol.L^{-1}) 25mL $\bar{X}_i \pm t_{0.05/2,\ n-1}\ \sigma_{n-1}/\sqrt{n}$
		[acid]mMol.L^{-1}							
		0	0.02	0.04	0.06	0.08			
		[H_2SO_4] mMol.L^{-1}							
1	Loba chemie India 98%	155	200	330	450	600	119±83.21+5700±1698.7[H_2SO_4] mMol.L^{-1}	0.9871 0.9744 97.44	0.021 52.5±4.59
		25	35	53	68	89	21.8±8.05+805±164.06[H_2SO_4] mMol.L^{-1}	0.9939 0.9878 98.78	0.027 67.5±3.11
2	THOMAS BAKER India	[Tartaric acid] mMol.L^{-1}					154±147.33+7000±3007.49 [Tartaric acid] mMol.L^{-1}	0.9737 0.9481 94.81	0.022 55.0±2.43
		160	280	400	660	670			
		16	20	32	41	57	12.6±8.75+515±178.79 [Tartaric acid] mMol.L^{-1}	0.9826 0.9655 96.55	0.024 60.0±2.21

ŷ: Estimated response 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, $t_{0.05/2,\ 2=4.303.}$

Paired t-test was used as shown in scheme 2 which shows a comparison- treatment of data was studied at two different paths.

First path : A comparison between two methods of analysis (scheme 2A) (i.e. Laser diode fluorimeter with pH-meter) as shown in table 14. B which based on the assumption:

Null Hypothesis $H_o : \mu_{\text{Laser diode fluorimeter}} = \mu_{\text{pH-meter}}$
Against
Alternative Hypothesis $H_1 : \mu_{\text{Laser diode fluorimeter}} \neq \mu_{\text{pH-meter}}$
A t-value for n-1 degree of freedom (i.e: $t_{0.05/2,\ n-1}$,12.706) . So, any value of $t_{calculated}$ less than 12.706 should lead to accept H_o i.e: there is no significant difference between the two methods of analysis . Calculated t-value = 2.00 for n-1 at α 0.05 (95%) which is indicate that 2.00< 12.706. Therefore ; H_o is accepted in favor of H_1 as shown that in table 14.B (column7).
This indicate that there is no significant difference between the means of the two different methods for analysis different acids. Therefore , we are going to **the second path** (scheme 2.B) for the calculation paired t-test to distinguish between the two different acids, and to explain if there were a significant difference in the source of H_3O^+ generation from different acids (i.e: , H_2SO_4 & tartaric acid) on the basis of that , the mean value of the obtained concentration:
(Null hypothesis) $H_o : \mu_{\text{Sulphuric acid}} = \mu_{\text{Tartaric acid}}$
Against
(Alternative hypothesis) $H_1 : \mu_{\text{Sulphuric acid}} \neq \mu_{\text{Tartaric acid}}$
From the results tabulated in table 14.B (column 11) shows that t_{cal} (0.5) < t_{tab} (12.706) , and on this basis there is no significant difference between different acids concerning on the amount of librated H_3O^+ ion. Since there is a constant concentration of I^-, IO_3^-, which in turn to form a constant I_2 whatever H_3O^+ sources.



Scheme 2- Paired t-test for the new adopted method (Laser diode fluorimeter)and pH-meter method for the determination of oxonium ion using two different approaches : A-represent comparison of the two methods data ; B- represent if there is no significant difference between two methods then the test is conducted between the two different acids data

Table 14-B: Paired t-test for comparison between two methods and two different acids (50mMol.L^{-1})

Type of method	Practically concentration mMol.L^{-1}		Type of pair									
			Laser diode fluorimeter-pH-meter					H_2SO_4- Tartaric acid				
	H_2SO_4	Tartaric acid	X_1d	x_{1d} (σ_{n-1})	N_1 df	$t_{cal}= \bar{x}_d\sqrt{n}/\sigma_{n-1}$	t_{tab}	X_2d	x_{2d} (σ_{n-1})	N_2 df	$t_{cal}= \bar{x}_d\sqrt{n}/\sigma_{n-1}$	t_{tab}
Laser diode fluorimeter	52.5	55.0	-15	-10 (7.07)	2	|-2|<<12.706 No significant difference between two methods		-2.5	2.5 (7.07)	2	0.5<<12.706 No significant difference between acids	
pH-meter	67.5	60.0	-5		1			7.5		1		

X_1d: different between Laser diode fluorimeter method with pH-meter, X_2d: difference between sulphuric acid with tartaric acid , $\bar{x}_{1d}$: average of difference between Laser diode fluorimeter method with pH-meter method , $\bar{x}_{2d}$: average of difference between sulphuric acid with tartaric acid, $t_{tab}=t_{0.05/2,\ n-1}$ =12.706 (for n=2) for paired t-test,t_{cal}= value of t-calculated for paired test, N_1: no.of methods ,N_2 : no. of acids, df: degree of freedom, σ_{n-1} : standard deviation

Conclusion

The newly method for the determination of oxonium ion is simple, rapid and sensitive. The method is based on reaction between oxonium ion and mixture of iodide and iodate ions to librated free iodine that quench of fluorescien solution (continuous fluorescence). The new method can be used to determine of oxonium ion in pure and commercial samples.

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