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Research Article

DETERMINATION OF IODIDE AND IODATE IONS USING QUENCHING FLUORESCENCE SYSTEM I^-- IO_3^--H_3O^+ VIA FLUORESCEIN SODIUM SALT MOLECULE

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ABSTRACT

A simple, rapid and sensitive method for the determination of iodide and iodate ions using laser diode fluorimeter-flow injection analysis via liberated of 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 irritated by laser source at 405nm.Chemical and physical parameters of this system were investigated. A linear calibration graph of 0.1-10 and 0.03-9mMol.L^{-1} were obtains for IO_3^- and I^- respectively, with L.O.D was 74.9ng/sample and 5.146ng/sample for IO_3^- and I^- respectively. Repeatability (RSD%) less than 1 was obtained . The proposed method was compared with standard method. By using paired t-test it was shown that there was no significant difference between the proposed method and official method and on this basis the new method can be accepted as an alternative analytical method.

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

1. INTRODUCTION

Iodine is an important micronutrient essential for the production of thyroid hormones that are involved in the regulation of many key biochemical reactions[(1)]. The deficiency of iodine can result in brain damage, mental retardation, and endemic goiter. Deficiency of iodine causes serious delay in neurological development. On the other hand, an excess of iodine or iodide can cause goiter and hypothyroidism. Table salts are iodized with iodate or iodide to serve as a source of iodine. The iodized salt is recognized as the method of choice and the most successful strategy for the prevention of iodine deficiency disorders. Most of the iodine in nature is found in marine sediment in the form of iodine salts. The most common forms of natural iodine in the diet are iodide and iodate, with additional iodized organic compounds providing a small fraction of bioavailable iodine[(2,3)]. There are many techniques reported for the determination of iodide and iodate ions in various samples include flow injection analysis [(4,5)], spectrophotometer [(6-10)], chromatography [(11,12)] and pology[(13)].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[(14,15)].This method combined with flow injection analysis technique and has been used for determination of iodate and iodide ion via the fluorescence quenching system I^--IO_3^--H_3O^+ using fluorescein salt as a fluorophore molecule via laser diode fluorimeter.

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 injection manifold reaction coupled with laser diode fluorimeter (blue purple 405nm-green 532nm) as shown in fig.1.A was used for the determination of oxoniumion 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 (2mMol.L^{-1} ,4mMol.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 (100mMol.$L^{-1}$$I^-$-50mMol.$L^{-1}$$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 fig.1.B.

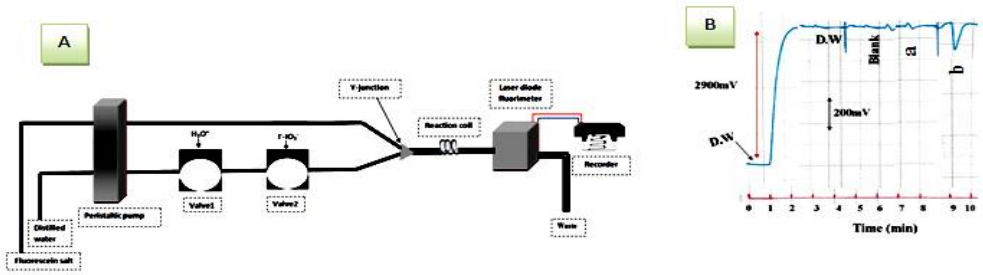
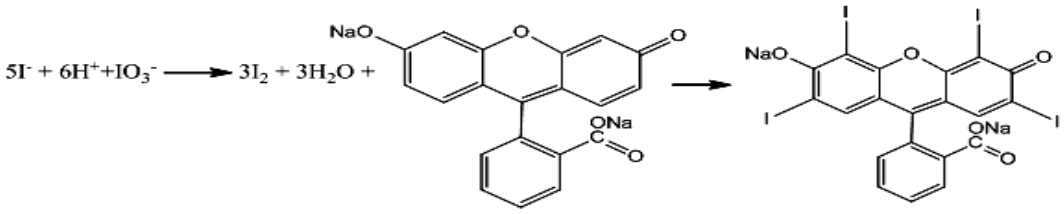


Fig. 1: A- Schematic diagram of flow injection analysis coupled with laser diode fluorimeter used the determination of iodide and iodate ions B- Response profile of 5mMol.L^{-1} fluorescein salt solution using quenchingSystem :a- 100mMol.$L^{-1}$$I^-$-50mMol.$L^{-1}$ IO_3^--2 mMol.L^{-1}HCland b- 50mMol.$L^{-1}$$I^-$-10mMol.$L^{-1}$ 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.



Fluorescein salt Erythrosine $C_{20}H_6I_4Na_2O_5$

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

3 .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).

3.1Chemical variables effect

3.1.1Fluorescein 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. Table1 was obtained explaining the increase in the continuous fluorescence with increases of fluorescein salt concentration and increases of quenching fluorescence response as shown in fig.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.

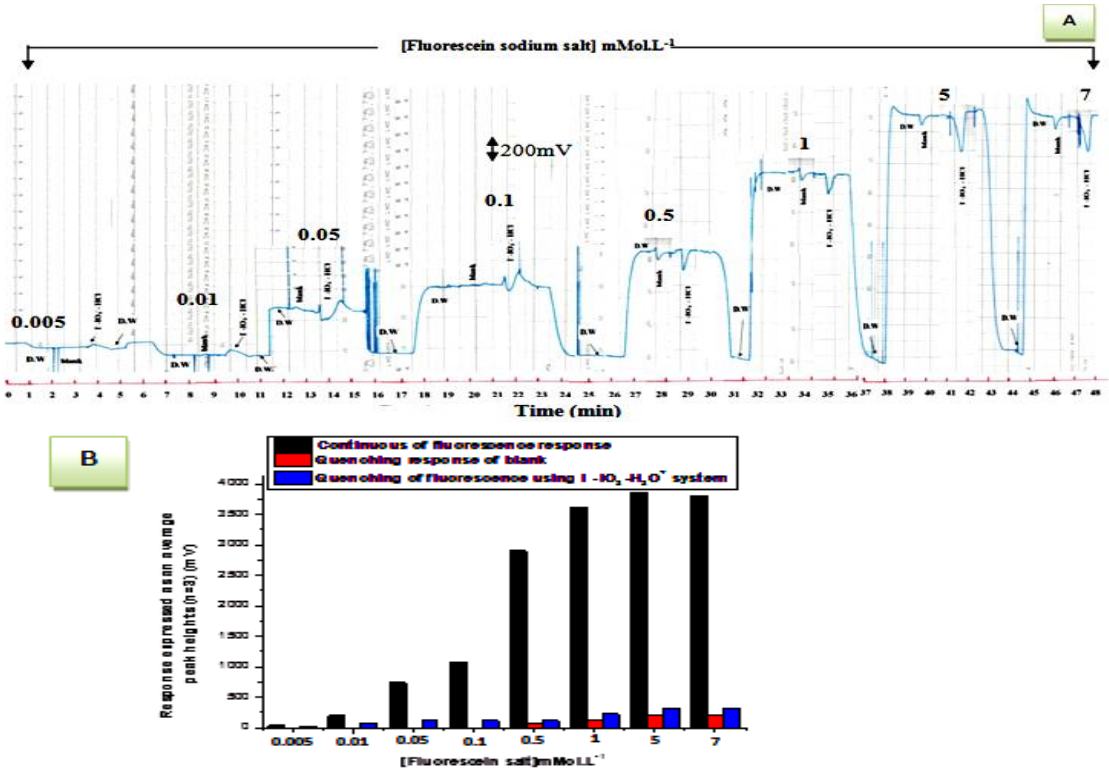


Fig. 2 : A- Response profile for eight concentrations of fluorescein salt solution B- Effect of fluorescein salt concentration on : response height of continuous fluorescence , quenching of fluorescence by : blank (I^--IO_3^-) and I^--IO_3^--H_3O^+ system

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

3.2 Physical parameter

3.2.1 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 fig.3.A,B and table 2, 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 fig.3.B. 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.

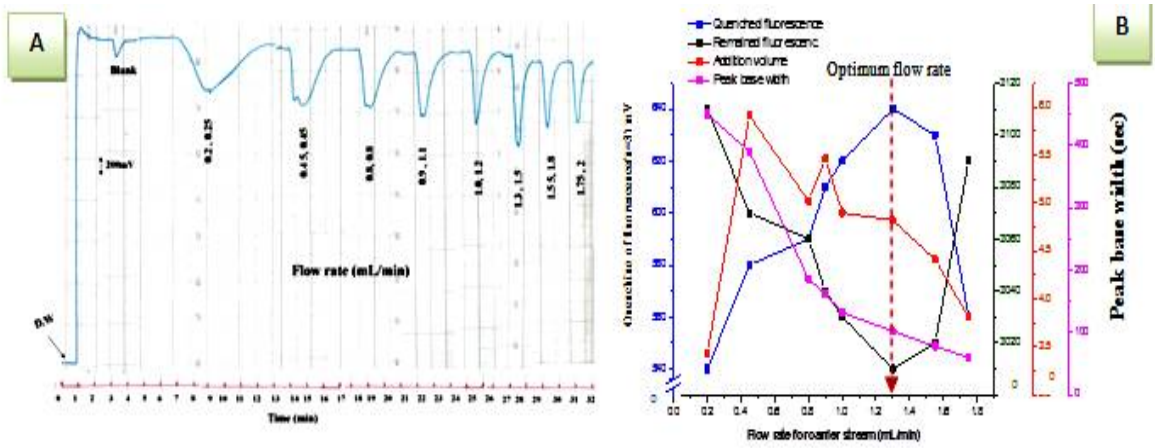


Fig. 3: Effect of variation of flow rate(mL/min) on: A-Response profile, B-Quenched, remained of fluorescence,addition volume and peak base width in mV

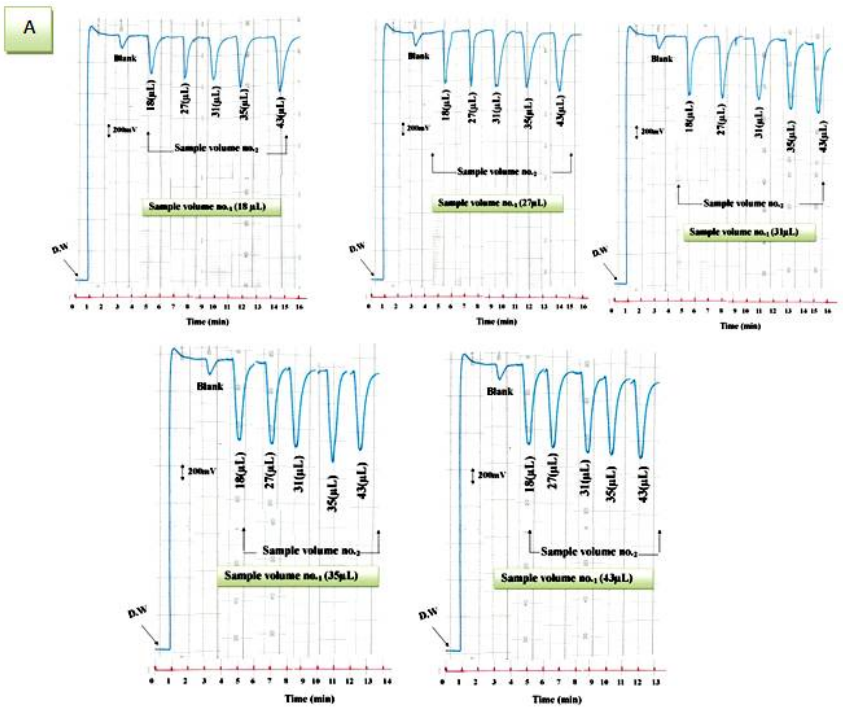
Table 2: Effect of the variation of flow rate (mL/min) on the fluorescence response (total, quenched and remained) using I^--IO_3^--H_3O^+ system

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 $\bar{y}_Q$ (n=3)mV	Remained fluorescence $\bar{y}_{R1}$(n=3)mV	Δt_b (sec)	t(sec)	V_{final} (mL)	Concentration in mMoLL^{-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

3.2.2 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 3 . 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 fig.4.A,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.



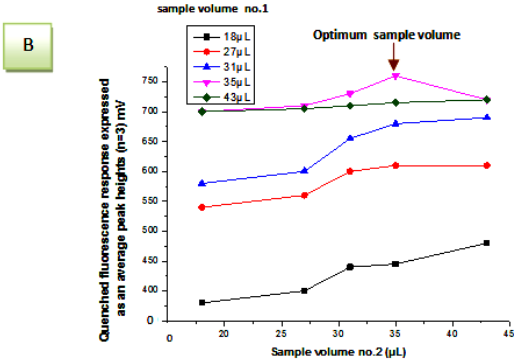


Fig. 4: Effect of different sample volume no.$_1$& no.$_2$ on : A-Sample of response profile , B-Quenched fluorescence response expressed as an average peak heights (mV) using laser diode fluorimete

Table 3: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	27	31	35	43
18	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

3.2.3 Study of the mixing pattern in two valves system for the formation of iodine molecule and effect of reactant on the quenched fluorescence

The possible combination of the chemical reactants at valve no.$_1$ and valve no.$_2$ to the formation of iodine molecule to quench fluorescein salt solution for the determination of iodide and iodate ions was studied. Three chemicals reactant are involved in the reaction ,i.e.the iodide ion, iodate ion,and the acidic medium (oxonium ion). The results obtained are shown in fig.5 and tabulated in table 4.

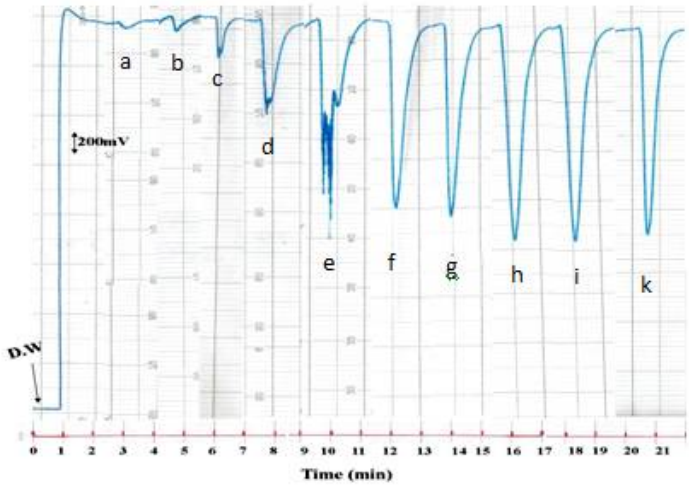
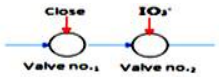


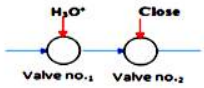
Fig. 5: Effect of chemical reactants & order of mixing in two valves on response profile of:

a :close valve no.1 and open valve no.2 (IO_3^-)

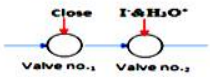


b: close valve no.1 and open valve no.2 (I^-)

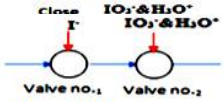
c: close valve no.2 and open valve no.1 (H_3O^+)



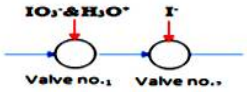
d: close valve no.1 and open valve no.2 (I^-& H_3O^+)



f:open valve no.1(I^-) and open valve no.2(IO_3^-& H_3O^+) sequentially

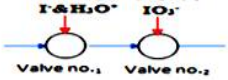


g: open valve no.1(IO_3^-& H_3O^+) and open valve no.2(I^-) sequentially



h: open valve no.1(IO_3^-) and open valve no.2(I^-& H_3O^+) sequentially

i: open valve no.1(I^-& H_3O^+) and open valve no.2 (IO_3^-) sequentially



k: open valve no.1(H_3O^+) and open valve no.2 (I^-&IO_3^-) sequentially

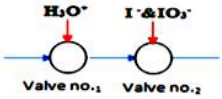


Table 4: Effect of mixing order in two valves of chemical reactants on the total and remained fluorescence response for the determination of iodide and iodate ions

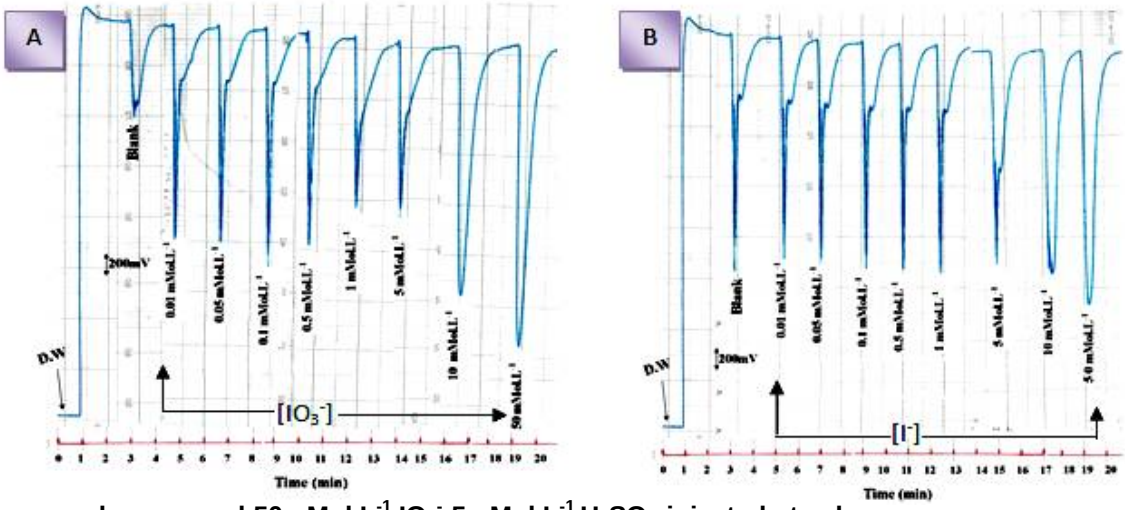
Order mixing in two valves for the carrier stream line	Total quenched fluorescence expressed as an average peak heights (n=3) $\bar{y}_i$ in mV	RSD%	Confidence interval of the average response (95% confidence level) $\bar{y}_{i(mV)} \pm t_{0.05/2,\, n-1} \sigma_{n-1} / \sqrt{n}$	Remained fluorescence $\bar{y}_{Ri}$ (n=3)mV
close valve no.$_1$ and open valve no.$_2$ (IO_3^-) (a)	60	3.33	60±4.96	3740
close valve no.$_1$ and open valve no.$_2$ (I^-) (b)	120	1.26	120±3.76	3680
close valve no.$_2$ and open valve no.$_1$ (H_3O^+) (c)	230	1.09	230±6.23	3570
close valve no.$_1$ and open valve no.$_2$ (I^-& H_3O^+) (d)	1200	0.2	1200±5.96	2600
close valve no.$_1$ and open valve no.$_2$ (IO_3^-& H_3O^+) (e)	2000	0.15	2000±7.45	1800
open valve no.$_1$(I^-) and open valve no.$_2$(IO_3^-& H_3O^+) (f)	2250	0.08	2250±4.47	1550
open valve no.$_1$(IO_3^-& H_3O^+) and open valve no.$_2$(I^-) (g)	2300	0.17	2300±9.71	1500
open valve no.$_1$(IO_3^-) and open valve no.$_2$(I^-& H_3O^+)(h)	2400	0.11	2400±6.56	1400
open valve no.$_1$(I^-& H_3O^+) and open valve no.$_2$(IO_3^-) (i)	2460	0.15	2460±9.17	1340
open valve no.$_1$(H_3O^+) and open valve no.$_2$(I^- &IO_3^-) (k)	2010	0.10	2010±4.99	1790

Response of continuous fluorescence : 3800mV

Previously the oxonium ion was injected in valve no.$_1$ while I^-&IO_3^- was injected via the valve no.$_2$ (table 4, pattern-k), thus will lead to the formation of I_2 and was determined through quenching of the fluorescence induced by 405nm laser beam. Using the same pattern with a slight modification i.e. thus injecting IO_3^- in valve no.$_1$ while valve no.$_2$ will introduce I^-&H_3O^+. High blank values was obtained

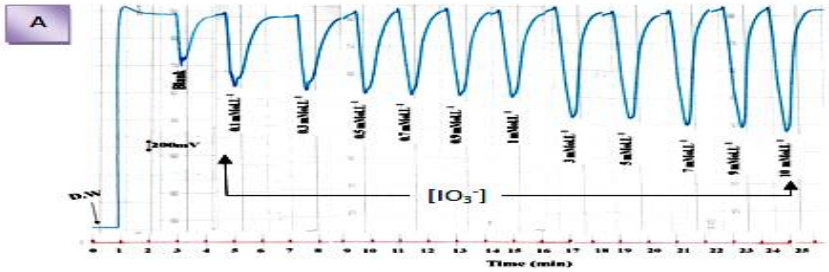
(see pattern-h) and this effect was study by preparing a serious of solutions with range (0.01-50mMol.L^{-1}) of IO_3^- at valve no.$_1$; while mixing of I^-(100mMol.L^{-1})-H_2SO_4 (5mMol.L^{-1}) at valve no.$_2$, the response profile as shown in fig.6.A. From this study was observed that the effect of blank (I^--H_3O^+) decreased at high concentration of IO_3^- (i.e more than 10mMol.L^{-1}). This was lead to change the pattern slightly by using the same original way of completing the reaction i.e injecting H_3O^+ in valve no.$_1$while injecting constant concentration of I^- with variable IO_3^- concentration in valve no.$_2$ .This pattern mixing give low background as well as lower blank values. This mentioned procedure was adopted for the determination of IO_3^-. Reversing through the use of variable I^- with constant concentration of IO_3^- in valve no.$_2$ while valve no.$_1$ will supply the H_3O^+ necessary lead to high blank level value. The effect of blank was study by preparing a series of iodide ion solutions having the range of concentration of 0.01-50mMol.L^{-1} injected at valve no.$_1$ and mixture of 50mMol.L^{-1} of IO_3^--5mMol.L^{-1} of H_2SO_4 injected at valve no.$_2$ .Fig.6.B shows a response profile, in which that, the increase of blank (i.e.IO_3^-- H_2SO_4) at low concentration of I^- compared with high concentration of I^- (10mMol.L^{-1}) .This study lead to changing the pattern of introducing the chemical used. Through the use of IO_3^- in valve no.$_2$ while introducing constant concentration of H_3O^+ and available concentration of I^- via the valve no.$_1$ lead to low level of blank (see pattern-a). On this basis I^- was determined in the range of 1µMol.L^{-1}-70mMol.L^{-1}(section 5.1).

Fig. 6: A- Variation of iodate ion concentration on the response profile when IO_3^- injected at valve no.$_1$ and 100mMol.L^{-1} I^--5mMol.L^{-1} H_2SO_4 injected at valve no.$_2$
B- Variation of iodide ion concentration on the response profile when I^- injected at valve no.$_1$ and 50mMol.L^{-1} IO_3^--5mMol.L^{-1} H_2SO_4 injected at valve no.$_2$



4.Study of the variation of iodate ion concentration with fixing concentration of iodide ion on the quenched fluorescence response using I^-- IO_3^--H_3O^+ system

A series of iodate ion solutions having the concentrations of 0.01-100 mMol.L^{-1}mixing with constant concentration of I^- **(**100mMol.L^{-1}) using 35µL as an injected sample volume at valve no.$_2$ while 5mMol.L^{-1} of H_2SO_4(35µL) injected at valve no.$_1$. Fig.7 .A shows a response profile and height for each iodate ion concentration. A correlation coefficient (r), coefficient of determination (r^2) and the percentage linearity (%r^2), all these of the linear regression analysis curve were summarized in table5 as shown in fig.7.B.This method compared with classical method using spectrophotometric measurements(iodate ion spectrophtometrically determined at UV absorption maxima at 352nm by reaction with iodide ion (0.2Mol.L^{-1}) in the presence of phosphoric acid (1Mol.L^{-1})[6]



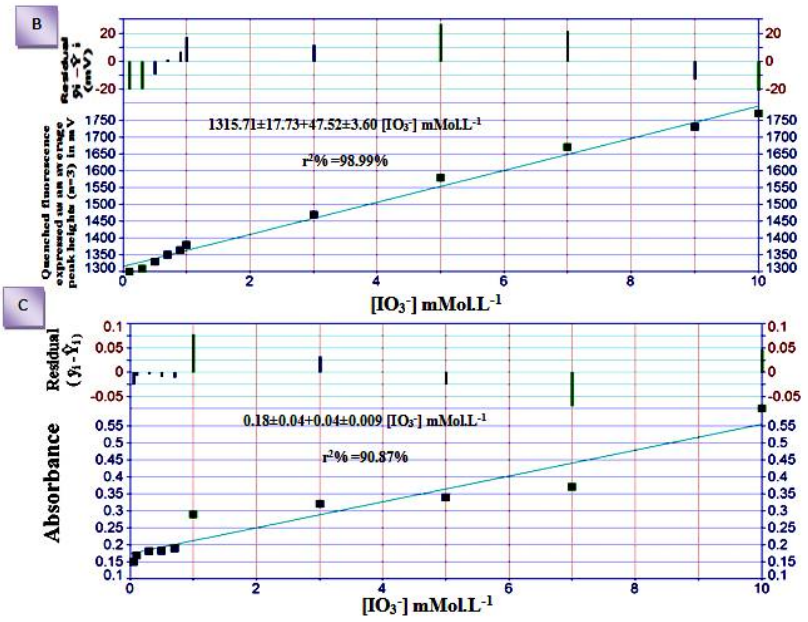


Fig. 7: Linear calibration graph for the variation of IO_3^- on: A-response profile, B-Quenched fluorescence response expressed as an average peak heights (mV)C-Calibration graph for the variation of IO_3^- concentration versus absorbance using spectrophotometer

Table 5: Summary of linear regression equations for the variation of IO_3^- concentration with total, quenched and remained fluorescence response using laser diode fluorimeter and using classical method via UV-spectrphotometer method

Measured $[IO_3^-]$ $mMol.L^{-1}$	Linear dynamic range $mMol.L^{-1}$	Type of measurement	$\hat{y}=(a\pm S_a t)+(b\pm S_b t)$ $[IO_3^-]mMol.L^{-1}$ at confidence level 95%, n-2	r r^2 $r^2\%$	t_{tab} at 95% confidence level, n-2	Calculated t-value $t_{cal}=\frac{\lvert r\rvert\sqrt{n-2}}{\sqrt{1-r^2}}$
0.01-100	n=11 0.1-10	Total quenched fluorescence	1545.71±17.73+47.52±3.60 $[IO_3^-]mMol.L^{-1}$	0.9949 0.9899 98.99	2.262<<29.85	
		Quenched fluorescence	1315.71±17.73+47.52±3.60 $[IO_3^-]$ $mMol.L^{-1}$			
		Remained fluorescence	2254.29±17.73-47.52±3.60 $[IO_3^-]$ $mMol.L^{-1}$			
0.01-100	n=10 0.05-10	Absorbance	0.18±0.04+0.04±0.009 $[IO_3^-]mMol.L^{-1}$	0.9533 0.9087 90.87	2.306<<8.93	

ŷ: estimated response (mV) for n=3 expressed as an average peak heights of linear equation of the form ŷ= a+bx or absorbance, $[IO_3^-]$: iodate ion concentration ($mMol.L^{-1}$), r :correlation coefficient, r^2:coefficient of determination, $r^2\%$: linearity percentage.

4.1 Limit of detection (L.O.D)

Limit of detection (lowest concentration in calibration graph) for iodate ion (IO_3^-) was calculated through using two approach as tabulated in table 6 via injected sample volume of 35µL.

Table 6: Limit of detection for iodate ion using quenching fluorescence system I^-- IO_3^--H_3O^+

Practically based on minimum concentration in calibration graph (0.01 $mMol.L^{-1}$)	Theoretical based on the value of slope $x=3S_B/slope$
74.9 ng/ Sample	283.71ng/Sample

X= value of L.O.D. based on slope , S_B= standard deviation of blank repeated for 13 times .

4.2. Repeatability

A repeatability of the proposed method was studied at fixed concentration of iodate ion mainly two concentrations were used (0.5 and 7mMol.L^{-1}) using the optimum parameters. A repeated measurements for eight successive injections were measured as shown in fig.8 and the obtained results is tabulated in table 7. A value of relative standard deviation less than 1% indicate clearly the high efficiency of the new applied method.

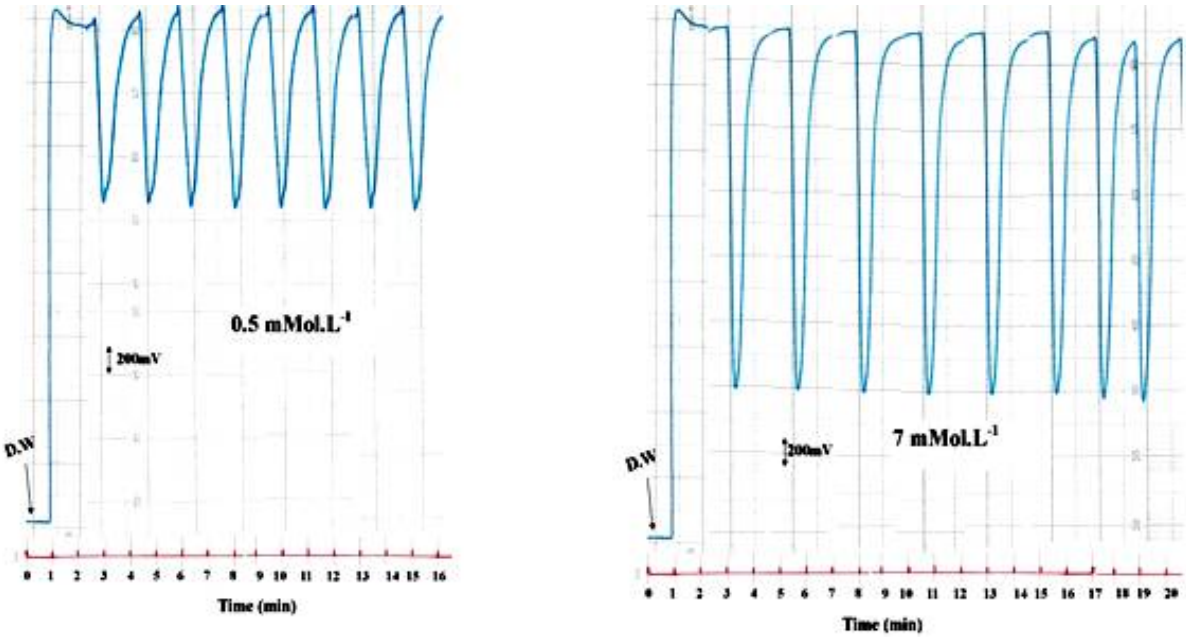


Fig. 8: Response profile of repeatability of iodate ion (0.5mMol.L^{-1} and 7mMol.L^{-1})

Table 7: Repeatability for the response to estimate IO_3^- using quenched fluorescence systemI$^-$- IO_3^--H_3O^+ at optimum parameters

Concentration mMol.L^{-1}	Average of total quenched fluorescence expressed as an average peak heights $\bar{y}_i$ in mV	Quenched fluorescence $\bar{y}_{Oi}$(n=8)mV	RSD%	Confidence interval of the total average response (95% confidence level) $\bar{y}_{i(mV)} \pm t_{0.05/2,\ n-1}\ \sigma_{n-1}/\sqrt{n}$
0.5	1550	1320	0.43	1550±5.57
7	1940	1710	0.75	1940±12.17

Response of continuous fluorescence=3800mV, Response of blank : 230mV,$t_{0.05/2,\ 7=2.365}$

Number of injection = 8

5.Study of the sample volume for the determination of iodide ion using quenched fluorescencesystem I$^-$- IO_3^--H_3O^+

Using the optimum parameters achieved in previous section , the variation of sample volume at valve no.$_1$ (18-45µL) with variation of sample volume at valve no.$_2$ (18-43µL) was studied when mixing solution (100mMol.L^{-1}I$^-$-5mMol.L^{-1}H_2SO_4) injected at valve no.$_1$ and 50mMol.L^{-1} IO_3^- injected at valve no.$_2$ using sequential open valves mode. The plot of change in sample volume of two valves versus quenched fluorescence response is shown in fig.9 and table 8. It was noticed that an increase of sample volume up to 31µL of injection valve no.$_1$ (43µL of sample volume no.$_2$) led to significant increase in response height of the quenched fluorescence response , while a larger sample volume (i.e., more than 31µL at valve no.$_1$ and 43 µL at valve no.$_2$) it gave a wider in Δt_b which might be attributed to the continuous relatively longer time duration of quenched fluorescence response segment in front of detector. On this basis 31µL at valve no.$_1$ and 43 µL at valve no.$_2$ was chosen as an optimum sample volumes of both valves.

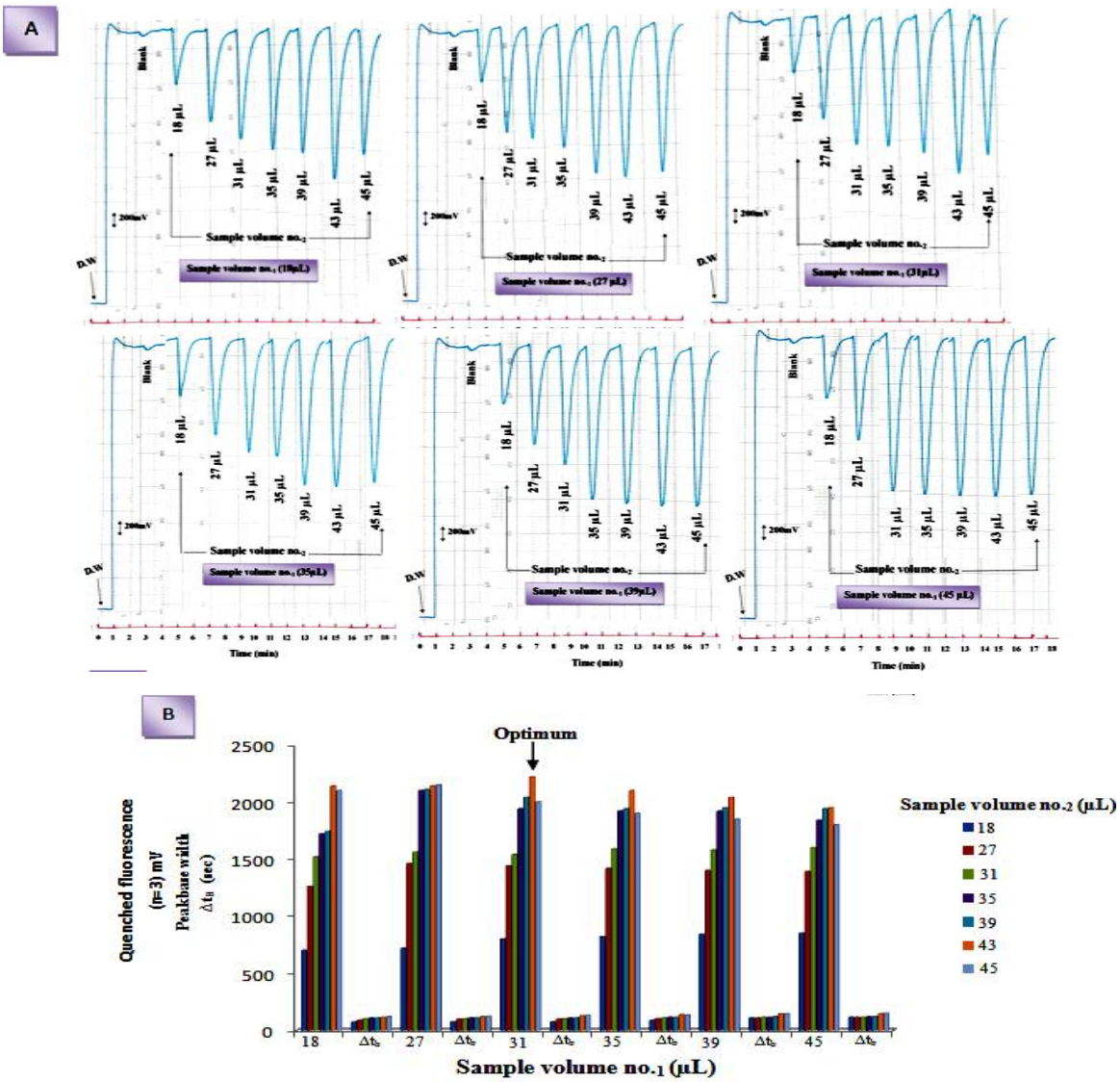


Fig. 9: Variation of sample volume segment of I^--H_2SO_4 at injection valve no.$_1$&valve no.$_2$of IO_3^-on :

A-Response profile

B-Quenched fluorescence response and peak base width (Δt_b)

Table 8: Effect of the variation of sample volume in two valves on the measurements of quenched fluorescence response and Δt_B for the determination of iodide ion using quenched fluorescencesystem I^-- IO_3^--H_3O^+

Sample volume no.-1 (µL)	Sample volume no.-2 (µL)						
Quenched fluorescence $\bar{y}_{Qi}$ (n=3)mV / Peak base width Δt_b (sec)	18	27	31	35	39	43	45
18	700 / 72	1260 / 90	1520 / 102	1720 / 108	1740 / 108	2140 / 114	2100 / 120
27	720 / 74	1460 / 96	1560 / 102	2100 / 108	2110 / 108	2140 / 120	2150 / 124
31	800 / 74	1440 / 96	1540 / 102	1940 / 108	2040 / 108	2220 / 126	2000 / 130
35	820 / 90	1420 / 102	1590 / 108	1920 / 114	1940 / 114	2100 / 138	1900 / 136
39	840 / 108	1400 / 108	1580 / 114	1920 / 114	1950 / 120	2040 / 144	1850 / 146
45	850 / 114	1390 / 114	1600 / 114	1840 / 120	1940 / 120	1950 / 146	1800 / 150

Response of continuous fluorescence=3830mV, Response of blank : 60mV

5.1Variation of total , quenched and remained of the fluorescence response versus concentration of iodide ion

A series 0.001-70mMol.L^{-1}of iodide solutions with constant concentration of sulphuric acid 5mMol.L^{-1} was prepared using the optimum parameters arrived in previous sections. Table 9 tabulated all the data obtained i.e; the straight line equation for the three types of measurements : total ,quenched and remained of fluorescence and shows correlation coefficient , the coefficient of determination , linearity percentage and the calculated t-value at 95% confidence level of 11.59 which larger than tabulated t-value indicating clearly that the linearity against non-linearity is accepted.Fig.10.A shows the response profile obtained. It can be seen, that there is an increase in the quenched fluorescence with increasing the iodide concentration up to 9mMol.L^{-1}. Larger than 9mMol.L^{-1} cause a decrease in r^2% and deviation from linear regression equation which might be attributed to increase the amount of I_3^- in front of the detector, thus does not affect on the quenching of fluorescence . The comparison with classical spectrophotometric method (the iodide ion was oxidized with hypochlorite 6ppm to form iodine, which then reacted with starch 0.1% to form a blue starch-iodine complex and measured at λ_{max}=618nm , optimum time for complex formation and oxidation was 15minutes) [7], the results obtainedwas tabulated in table 9. The linear calibration range was obtained is shown in fig.10,B,C for the adopted and classical method.

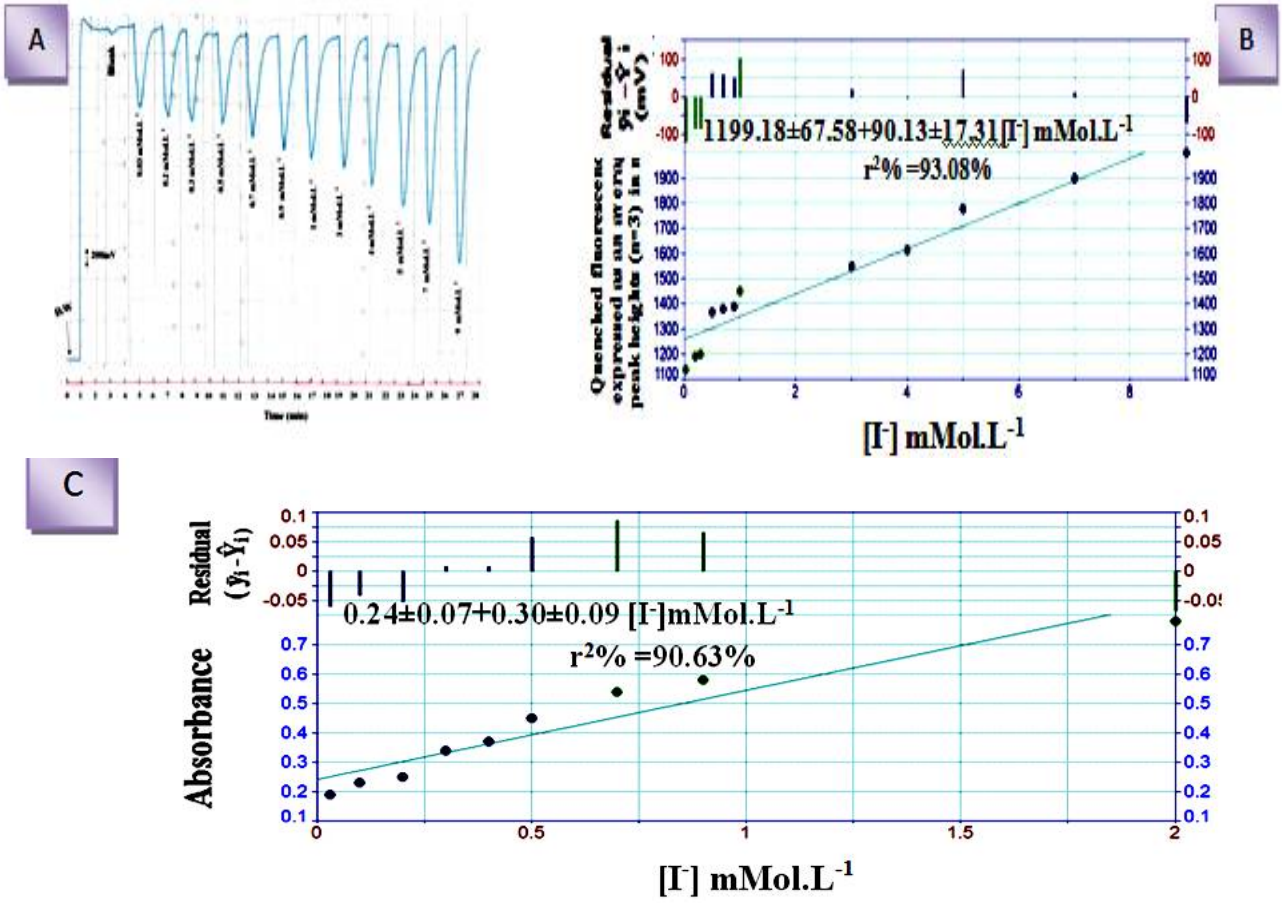


Fig. 10: Calibration graph for the variation of iodide ion on : A- Response profile, B- Quenched fluorescence response expressed by linear equation using laser diodefluorimeter and C-Linear regression using spectrophotometer (starch-iodine complexspectrometrically measured at 618nm)

Table 9: Summary of linear equations for the variation of instrument response with iodide ion concentration using flow injection coupled with laser diode fluorimeter and classical method of spectrophotometer

Measured [I⁻] mMol.L^{-1}	Linear dynamic range mMol.L^{-1}	Type of measurement	ŷ=(a± S_at)+(b±S_bt) [I⁻]mMol.L^{-1} at confidence level 95%, n-2	r r^2 r^2%	t_{tab} at 95% confidence level, n-2	Calculated t-value $t_{cal}=\frac{\lvert r\rvert\sqrt{n-2}}{\sqrt{1-r^2}}$
0.001-70	n=12 0.03-9	Total quenched fluorescence	1259.18±67.58+90.13± 17.31 [I⁻]mMol.L^{-1}	0.9648 0.9308 93.08	2.228<<11.59	
		Quenched fluorescence	1199.18±67.58+90.13± 17.31[I⁻] mMol.L^{-1}			
		Remained fluorescence	2570.82±67.58-90.13±17.31 [I⁻] mMol.L^{-1}			
0.005-100	n=9 0.03-2	Absorbance	0.24±0.07+0.30±0.09 [I⁻]mMol.L^{-1}	0.9519 0.9063 90.63	2.365<<8.23	

ŷ: estimated response (mV) for n=3 expressed as an average peak heights of linear equation of the form ŷ= a+bx or absorbance , [I⁻] : iodide ion concentration (mMol.L^{-1}), r :correlation coefficient, r^2:coefficient of determination, r^2%: linearity percentage.

5.2 Limit of detection

Two different approaches were used, detection based on lowest concentration in the calibration graph, or detection based on the numeric value of slope. Table 10 tabulated all these calculation value of detection limit for 31µL sample volume.

Table 10: Limit of detection for iodide ion at optimum parameter, 31µL sample volume

Practically based on minimum concentration in calibration graph (0.001 mMol.L^{-1})	Theoretical based on the value of slope x=3S_B/slope
5.146 ng/ Sample	274.057ng/Sample

X= value of L.O.D. based on slope , S_B= standard deviation of blank repeated for 13 times .

5.3 Repeatability

The repeatability was studied via measurements of RSD% for some selected concentration of iodide ion (n=8). The response profile at concentration 0.7 and 7mMol.L^{-1} of eight successive injected sample measurement as shown in fig.11 and all results obtained were tabulated in table 11.

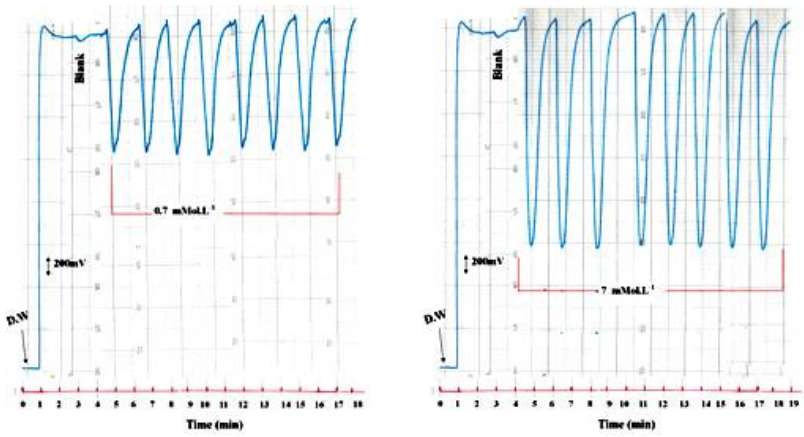


Fig. 11: Response profile for iodide ion using 0.7 and 7mMol.L^{-1}

Table 11: Repeatability of iodide ion results

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 total average response (95% confidence level) $\bar{y}_{i(mV)} \pm t_{0.05/2,\ n-1}\ \sigma_{n-1}/\sqrt{n}$
0.7	1340	1280	0.38	1340±4.26
7	1815	1755	0.20	1815±3.04

Response of continuous fluorescence=3830mV , Response of blank : 60mV, $t_{0.05/2,\ 7=2.365}$
Number of injection = 8

6.Application of the adopted methodology for the assay iodate and iodide ions

A- Iodate ion

flow injection analysis coupling with optimum parameters that achieved in previous sections for the quenching system I^--IO_3^--H_3O^+ which formed an iodine molecule species which quenched continuous fluorescence (fluorescein sodium salt solution). Iodate ion was tested via the use of standard addition method in two different commercial samples (Thomes baker, India and Hi media , India) . The standard addition method was applied by transferring 0.5mL of 50mMol.L^{-1} IO_3^- to each the five volumetric flasks (25mL), followed by the addition of 0,2,3,4 and 5mL of 50mMol.L^{-1} standard solution of IO_3^- to obtain the range of concentration (0-10mMol.L^{-1}) for the measurements by both methods (Laser diode fluorimeter and spectrophotometer).Fig.12 A_1,B_1 shows the response profile for this study and standard addition calibration graphs using laser diode fluorimeteras shown in fig.12.A_2 and B_2. The results obtained are summarized in table 12.

B-Iodide ion

The adopted method achieved in this work was used also for the analysis of iodide ion in three different of table salts and was compared by spectrophotometric method via the measurement of λ_{max} at 610nm. The standard addition method was applied by preparing a series of solutions from each table salt via transferring 10mL (0.1mMol.L^{-1}) to five volumetric flasks (25mL) followed by the addition of gradual volumes of standard iodide ion solution(50mMol.L^{-1}) (0,0.1,0.3,0.5 and 0.7mL) in order to have the concentration range from 0-1.4mMol.L^{-1} for the preparation of standard addition plot as shown in fig.12.C,D and E. The measurements were conducted by both methods were tabulated in table 12.

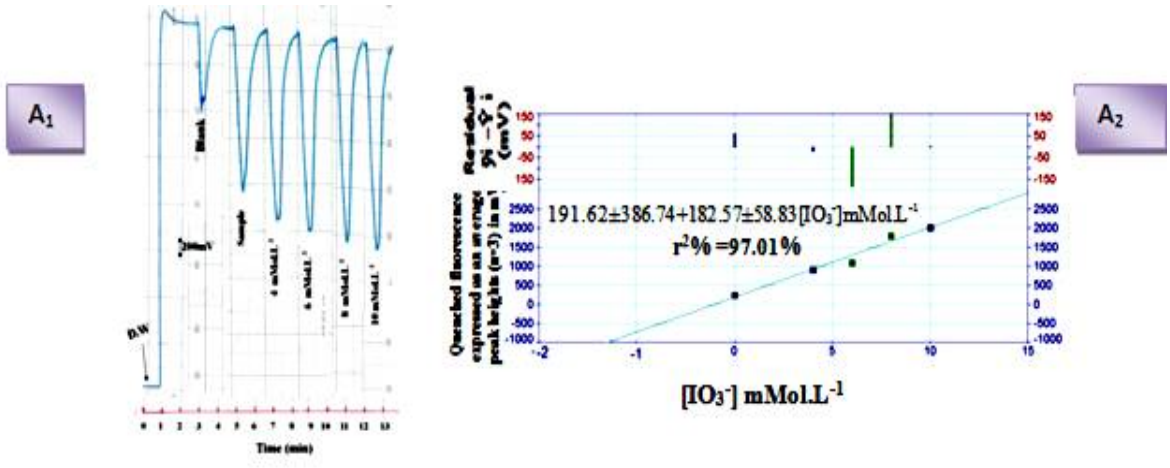


Fig. 12: A_1,A_2,B_1 and B_2: standard calibration graph and response profile of two commercial of iodate samples. C,D and E standard calibration graph of three table samples of iodide ion

Table 12: Results for the determination of iodate and iodide ions using standard addition, with two methods, laser diode fluorimeter and spectrophotometer method with paired t-test for comparison between two methods

Type of ion	No. of sample	Company and country of sample	Sample weight equivalent to 50mMol.L^{-1} for IO_3- and 0.1mMol.L^{-1} for I^- (g)	Laser Diode Fluorimeter / Spectrophotometer: $\hat{y}=a\pm S_a t+ b\pm S_b t$ [IO_3^-] mMol.L^{-1} at confidence level 95%, n-2	r / r^2 / r^2%	Practical concentration mMol.L^{-1} in 25mL	Practical weight $\overline{Wi} \pm 4.303\sigma_{n-1}/\sqrt{n}$ at 95% confidence interval	Paired t-test (comparison between two method): Xd	$\overline{x_d}$ (σ_{n-1}) N, df	$\frac{t_{cal}}{\overline{x_d}\sqrt{n}/\sigma_{n-1}}$	Sig. 2-tailed
KIO$_3$	1	Thomes Baker India KIO_3	0.2675	191.62±386.74+182.57±58.83[IO_3^-]mMol.L^{-1}	**0.9849 0.9701 97.01**	1.049 / 52.45±1.74	0.2806g±0.009	9.6	3.95 (7.99) N=2 df=1	0.699<< 12.706	0.612> 0.05 No sig.
				0.06±0.15+0.07±0.02 [IO_3^-] mMol.L^{-1}	**0.9836 0.9675 96.75**	0.857 / 42.85±1.49	0.2292g±0.008				
	2	Hi media India KIO_3		189.46±346.27+170.81±52.68[IO_3^-] mMol.L^{-1}	**0.9862 0.9726 97.26**	1.109 / 55.45±1.65	0.2967g±0.0008	-1.7			
				0.08±0.26+0.07±0.04[IO_3^-] mMol.L^{-1}	**0.9489 0.9006 90.06**	1.143 / 57.15±2.48	0.3058g±0.01				
						(0.1mMol.L^{-1}) 25mL					
KI	1	Al Mansour Iraq 0.008%KI	20.75	86.83±690.30+1701.83±842.12[I^-] mMol.L^{-1}	**0.9656 0.9324 93.24**	0.051 / 0.13±0.07	0.01%±0.005	0.02	0.0067 (0.0252) N=3 df=2	0.459 <<4.303	0.691> 0.05 No.sig
				0.02±0.2+0.46±0.24[I^-] mMol.L^{-1}	**0.9604 0.9223 92.23**	0.043 / 0.11±0.12	0.008%±0.009				
	2	الضحى المملكة العربية السعودية 50-80mg/Kg KI	33.20	95.61±597.79+1700.61±729.22[I^-] mMol.L^{-1}	**0.9738 0.9483 94.83**	0.056 / 0.140±0.03	70mg/kg±15.00	-0.01			
				0.03±0.20+0.51±0.25[I^-] mMol.L^{-1}	**0.9675 0.9360 93.60**	0.059 / 0.15±0.07	74mg/kg±34.53				
	3	Zer Turkey 25-40mg/Kg KI	66.40	74.56±585.32+1521.31±714.01[I^-] mMol.L^{-1}	**0.9689 0.9387 93.87**	0.049 / 0.12±0.02	30.75mg/kg±5.12	-0.03			
				0.03±0.15+0.52±0.19[I^-] mMol.L^{-1}	**0.9809 0.9622 96.22**	0.058 / 0.15±0.04	36.25 mg/kg ±9.67				

Xd: Difference between two method , □ d: difference mean , σ_{n-1}:Difference standard deviation , N= no. of sampl

From table 12 that the two iodate samples from different manufacturer were analysed by both spectrophtometric or via the newly developed method of assessment. The output come were subjected to a paired t-test[16,17] to evaluate if there were a difference in the mean of the both methods. Therefore the assumption were made at α= 0.05 level of significance.

Null hypothesis : $H_o = \mu_{spectrophotometer} = \mu_{New\ develop}$

or : $\mu_{spectrophotometer} - \mu_{New\ develop} = zero$

i.e: there is no significant difference between the means of the two methods while the alterative hypothesis.

Altertativehypothesis : $H_1 : \mu_{spectrophotometer} \neq \mu_{New\ develop}$

or : $\mu_{spectrophotometer} - \mu_{New\ develop} = zero$

At a significant level of α = 0.05 (confidence of 95%). Any value < 0.05 will reject Null hypothesis and accepted the alternative hypothesis. Reversing ; any value of significant > 0.05 will accept Null hypothesis processing the measurement data via SPSS-20 : Shows that a significance level of 0.612 was obtained thus indicating a well accepted H_o in favour of the H_1 . Therefore the new developed method can be accepted or can describe the range of assessment by fluorescence method as the spectrophotometric method.

On the same basis , three different samples from different manufacturer containing different amount of iodine as KI was subjected to the same treatment as in above and shows that : the Null hypothesis :

$H_o : \mu_{sample\ (1)} = \mu_{sample\ (2)} = \mu_{sample\ (3)}$

There is no significant difference between the means obtained from three different samples. This is clearly indicated, that both methods can not distinguish between different samples.

Against :

Alternative hypothesis : $H_1 : \mu_{sample\ (1)} \neq \mu_{sample\ (2)} \neq \mu_{sample\ (3)}$

There is a significant difference between two methods i.e: one or both methods can be distinguish between different samples.

Since, the significance value is 0.691>0.05; Null hypothesis will be accepted, i.e: favour H_o against H_1 at α = 0.05.

Therefore a final conclusion is that the newly developed fluorescence method based on quenched emissioned$\lambda_{excitation}$ = 405nm for fluorescence molecule can be used as an alternative assessment method for determination of I^- in different samples.

CONCLUSION

A laser diode fluorimeter-flow injection analysis procedure is proposed for the determination of iodate and iodide ion with application in the analysis of commercial and table salt samples. The method is based on liberated of iodine moleculefrom the I^--IO_3^--H_3O^+ reaction which react with fluorescein sodium salt solution causing to quench the fluorescence light (continuous fluorescence) when irritated by laser source at 405nm. The RSD% was < 1% and good agreements were observed for all samples, which is indication of satisfactorily accuracy of the proposed method. The standard addition method was used to avoid matrix effects. Statistical analysis for the results using t-test showed no significant difference at p=0.05 between the performance of the two methods as regards to accuracy and precision.

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