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Research Article

A NEW METHOD FOR THE DETERMINATION OF [H_3O^+] ION IN ORGANIC ACIDS VIA 532 NM SOLID STATE LASER DIODE WITH CONTINUOUS FLOW INJECTION

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ABSTRACT

The new method is rapid, sensitive and simple for the determination of Hydronium ion in organic acids using 532nm solid state laser diode with continuous flow injection analysis and using laser dye (Rhodamine6G) as a fluorescent host .The method was based on quenching of fluorescence for Rhodamine6G via organic acid. A liner range of organic acid was 7-2000mMol.L^{-1}forascorbic acid ($C_6H_8O_6$),7-1000mMol.$L^{-1}$for tartaric acid ($C_4H_6O_6$)and 10-1800mMol.$L^{-1}$for citric acid($C_6H_8O_7$),with correlation coefficient $r_=$ 0.9758, 0.9756 and 0.9802, successively the limit of detection(LOD)was323.356,344.450and 352.732µg/sample based on gradual dilution of lowest concentration in calibration graph. The new method can be accepted as an alternative analytical method.

Keywords: Organic acids (ascorbic acid, tartaric acid and citric acid), Dyes laser, Flow injection.

1. INTRODUCTION

Organic acid is an organic compound with acidic properties. The most common organic acids are the carboxylic acids, whose acidity is associated with their carboxyl group COOH. Most acids are weak acids. Examples of weak acids include acetic acid, formic acid , ascorbic acid and tartaric acid[1]. In general, organic acids are weak acids and do not dissociate completely in water. Lower molecular mass organic acids such as formic and lactic acids are miscible in water, but higher molecular mass organic acids, such as benzoic acid, are insoluble in molecular (neutral) form. On the other hand, most organic acids are very soluble in organic solvents. p-Toluenesulfonic acid is a comparatively strong acid used in organic chemistry often because it is able to dissolve in the organic reaction solvent[2]. Exceptions to these solubility characteristics exist in the presence of other substituents that affect the such as polarity of the compound[3,4].Simple organic acids like citric are used as rust removal, cosmetics and pharmaceuticals and food and drink,tartaric acid and its derivatives have a plethora of uses in the field of pharmaceuticals. Tartaric acid also has several applications for industrial use[5]. Some of the most commonly used methods for determination of organic acids include continuous flow injection[6],iodometric titration[7], quantitativemethods[8],high-performance liquid chromatography[9], HPLC/UV[10]. Laser dyes are large organic molecules and laser gain medium are organic dyes in solution of ethyl, methyl alcohol, deionized water or distilled water[11,12]. Organic dye lasers are capable of emission across a broad band width making them suitable for tunable lasers. There are several classes of laser dyes including polymethines (700 to 1500 nm),Xanthenes also known as Rhodamines (500 to 700 nm), coumarines (400 to 500 nm) and scintillator(320 to 400 nm) Dyes exhibit a very high degree of fluorescence, i.e., when the dye is exposed to ultraviolet light, it glows with characteristic color depending on the nature of the material[13,14].Dye lasers belong to the family of liquid lasers. The active material is a dye dissolved in a liquid solvent[15,16]. Flow injection analysis technique has been employed to automate a wide variety of chemical/biochemical analyses

since its invention in the early 1970[17,18]. Based on the injection of a liquid sample into a moving, non-segmented continuous carrier stream of a suitable liquid. The injected sample forms a zone, which is then transported toward a detector that continuously records the changes in absorbance, electrode potential, or other physical parameter resulting from the passage of the sample material through the flow cell[19].The important types of flow injection analysis technique are segmented flow injection analysis[20],continuous flow injection analysis[21], stopped-flow injection analysis[22],reverse flow injection analysis[23],sequential-flow injection analysis[24].The flow injection analysistechnique was used in many determination of pharmaceuticals or ions[25].

2. EXPERIMENTAL

2.1. Reagents and chemicals

A stock solution (0.01 Mol.L^{-1}) of Rhodamine6G ($C_{28}H_{31}N_2O_3Cl$,M.wt479.02 g.moL^{-1}) was prepared by dissolving 2.3951g in 500 ml of distilled water.A stock solutions of acids ascorbic acid (176.12 g.mol^{-1},HIMEDIA ,2Mol.L^{-1})tartaricacid(150.087g.mol^{-1},THOMAS BAKER, 2Mol.L^{-1}) and citricacid (192.12g.mol^{-1},HIMEDIA,2Mol.L^{-1}) was prepared bydissolving352.24 g,300.174 gand 384.24g respectively and complete the volume with distilled water to 1000 ml volumetric flasks.

2.2.Sample preparation of Vitamin C tablets

A batch of thirty tablets were weighted , crushed , grinded and sieved via (through) 200 mesh sieve. Each of the drug containing 500 mg of vitamin C (UK),were weighted:19.0985g which equivalent to 8.806g of active ingredient to obtain 500 mMol.L^{-1} using 532nm solid state laser diode and 0.0382g which equivalent to 0.0176 g of active ingredient to obtain 1 mMol.L^{-1} using UV-VIS spectrophotometer. The powder was dissolved in distilled water followed by filtration to remove any undissolved residue affecting on the response. The volume was completed with distilled water to 100 mL.

2.3.APPARATUS

Laser diode fluoroimeter is a homemade instrument that is capable in measuring fluorescence at 405(10mW),and 532nm(not less than 1000mW) laser diode. Both 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 a maximum radiation area for a flow cell having outside diameter 4mm inside diameter 2mm (path length for absorption of irradiation) is 90°.The schematic diagram in figure no.1 shows the system used ,which comprises the useperistaltic pump four channels, variable speed (Ismatec type ISM 796), A rotary 6-port injection valve(Rheodyne, U.S.A) with a sample loop(id 1 mm , Teflon, Variable length) used for sample injection .The output signals was recorded by x-t potentiometric recorder(KOMPENSO GRAPH C-1032) Siemens (Germany).UV-VIS Spectrophotometer digital double beam (type UV-1800 , Shimadzu, Japan)was also used to scan the spectrum of colored species using Quartz cell and compare with new methods(laser diode fluoroimeter) for determination ascorbic acid (vitamin C) in vitamin C tablets 500mg UK.

2.4.Methodology

A preliminary investigation of the use of twin laser diode fluoroimeter (i.e.; mainly 405nm and 532nm)each on a separate basis but in one instrument . 532nm laser irradiation source was the choice which coupled with flow injection analysis technique.A simplified flowgram as shown in figure no.(1). The manifold used was composed from one line.The carrier stream (Rhodamine6G) (1.1×10^{-4}Mol.L^{-1}) at 2 mL.min^{-1} flow ratewhich lead to the injection valve to react with the injected sample volume (459µL) organic acids (ascorbic, tartaric, citric acid)and carry the mixture tocomplete the reaction.Thesegment passes through flow measuring cell. The response profile of which was recorded on x-t potentiometric recorder to measure quenching of fluorescence expressed as peak height in mV.

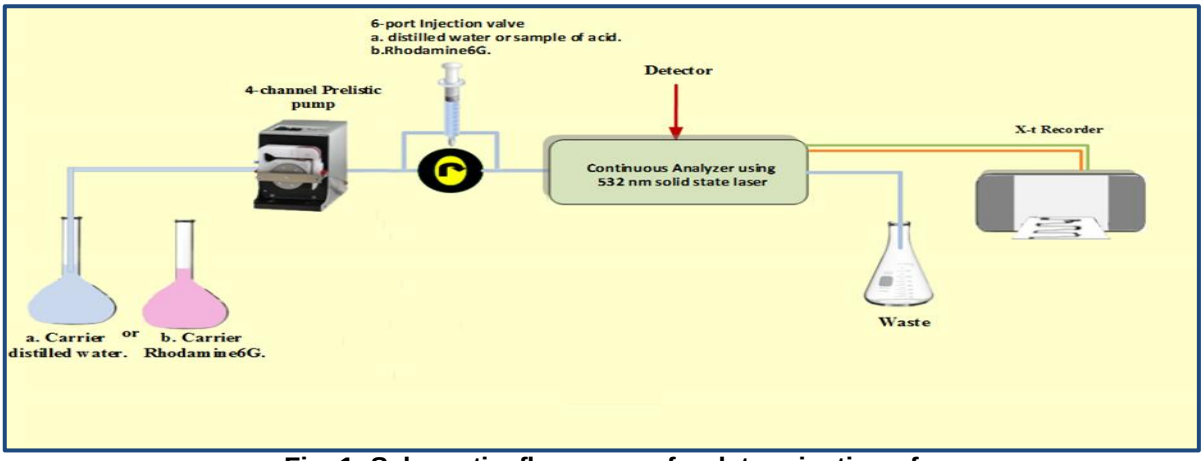

Fig. 1: Schematic flow gram for determination of organic acids via the use Rhodamine6G (laser dye)

3. Study of the optimum parameters for Determination of organic acids using 532nm solid state laser diode-continuous flow injection analyzer.

A series of experiments were conducted to establish the conditions for the production of maximum repeatable response with good sensitivity for Determination of $[H_3O^+]$ ion. The chemical variables such as concentration of fluorescent dye used (Rhodamine6G)and physical variables including, flow rate, sample volume and purge time were investigated respectivelyas shown in reference [26].

4.Calibration graph of organic acids

4.1.Calibration graph of Ascorbic acid ($C_6H_8O_6$)

A stock solution of ascorbic acid (2Mol.L^{-1}), via (176.12g) in distilled water brings him to a series of ascorbic acid solutions ranging from (5 to 2000) mMol.L^{-1} and injected at the established optimum condition(c.f. section 3). Since all data are involved with relatively good correlation value of $r_=$ 0.9758.These value will be regarded as the calibration graph to be used all over the research work calibration extend from 5 mMol.L^{-1} up to 2000 mMol.L^{-1} higher concentration was not used (i.e.; above 2000 mMol.L^{-1} ascorbic acid) to avoid any disadvantages might occur.Table no.1 show Scatter plot of measured data for ascorbic acid, while table no.2show all range that was used. Figure no.2 show calibration graph for ascorbic acid while a sample of fluorescence intensity vs time profile can be seen in figure no.(5).

Table 1: Scatter plot data for ascorbic acid (i.e.; all measured data were used)

Concentration of $C_6H_8O_6$ in distilled water (mMol.L^{-1})	$\bar{y}_i$ mV	S.D σ_{n-1}	RSD %	$\bar{y}_i \pm t$ SEM
5	10	0	0	5 ± 0
7	20	0	0	7 ± 0
9	30	0	0	9 ± 0
10	40	0	0	40 ± 0
50	200	0	0	200 ± 0
100	340	0	0	340± 0
200	418	2.89	0.69	418 ± 7.18
300	715	5	0.7	715 ± 12.42
400	777	5.77	0.74	777 ± 14.33
500	800	0	0	800 ± 0
600	897	5.77	0.64	897 ± 14.33
700	960	0	0	960 ± 0
800	1035	5	0.48	1035 ± 12.42
900	1075	5	0.47	1075 ± 12.42
1000	1233	5.77	0.47	1233 ± 14.33
1200	2240	0	0	2240 ± 0
1500	2580	0	0	2580 ± 0
1800	2625	5	0.19	2625 ± 12.42
2000	2655	5	0.19	2655 ± 12.42

SEM $=\sigma_{n-1}/\sqrt{n}$ Confidence interval of the mean at 95 for n-1$=\bar{y}_i \pm t$ SEM

Table 2: Range of the used concentration for Ascorbic acid

Range of used concentration (mMol.L^{-1})	Correlation coefficient (r)	r^2%
7-2000	0.9758	95.22

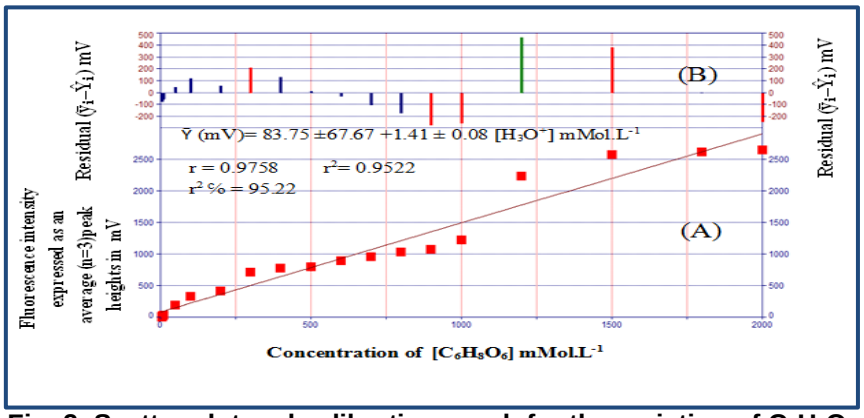

Fig. 2: Scatter plot and calibration graph for the variation of $C_6H_8O_6$ concentration on: A- Fluorescence intensity, B- residual ($\bar{y}_i$–$\hat{Y}_i$)

4.2.Calibration graph of Tartaric acid ($C_4H_6O_6$)

A series of tartaric acid solutions ranging from (7 to 2000) mMol.L^{-1} were prepared from a stock solution having the concentration of 2Mol.L^{-1} via (150.087g) in distilled water and injected at the established optimum conditions(c.f. section 3) . Table no.3 show Scatter plot of measured data for tartaric acid,while table no.4show all range that was used.Conducting the treatment of obtained data as shown in Figure no.(3)which shows the plot of all data without neglecting any measurement, for the concentration range (7-2000) mMol.L^{-1}. Linear regression for simple linear equation of the form $Y_{=}$ a $_{+}$bx gave a correlation coefficient of $r_{=}$ 0.8911 with an explained values for all data given to be 65.92 %. This is regarded a weak correlation and the equation used cannot describe more 2/3 of the data in order to improvethe correlation by canceling any measurement exceeding over 1000 mMol.L^{-1} i.e.; restricting the calibration to be extended from (7-1000) mMol.L^{-1} which gave a value of $r_{=}$ 0.9756 with an ability to explain about 95.18 % of the data taken by the chosen linear equation. Also a further restriction of calibration graph was carried out to be (10-1000) mMol.L^{-1} there was a little improvement achieved with $r_{=}$ 0.9800 and an explained value by the chosen equation by 96.04%. A comparison between the last two treatment dose not really gave any noticeable improvement in the linearity range. Therefore both calibration range can be used.A sample of fluorescence intensity vs time profile can be seen in figure no.(5).

Table 3: Scatter plot data for tartaric acid (i.e.; all measured data were used)

Concentration of $C_4H_6O_6$ in distilled water (mMol.L^{-1})	$\bar{y}_i$ mV	S.D σ_{n-1}	RSD %	$\bar{y}_i \pm t$ SEM
7	10	0	0	10 ± 0
9	15	0	0	15 ± 0
10	60	0	0	60 ± 0
50	240	0	0	240 ± 0
100	360	0	0	360 ± 0
200	517	5.77	1.12	517 ± 14.33
300	593	5.77	0.97	593 ± 14.33
400	657	5.77	0.88	657 ± 14.33
500	840	0	0	840 ± 0
600	1013	5.77	0.57	1013 ± 14.33
700	1053	5.77	0.55	1053 ± 14.33
800	1100	0	0	1100 ± 0
900	1140	0	0	1140 ± 0
1000	1300	0	0	1300 ± 0
1200	960	0	0	960 ± 0
1500	1025	5	0.49	1025 ± 12.42
1800	1120	0	0	1120 ± 0
2000	1175	5	0.43	1175 ± 12.42

SEM $=\sigma_{n-1}/\sqrt{n}$ Confidence interval of the mean at 95 for n-1$=\bar{y}_i \pm t$ SEM

Table 4: Range of the used concentration for Tartaric acid

Range of used concentration (mMol.L^{-1})	Correlation coefficient (r)	r^2%
7-2000	0.8119	65.92
7-1000	0.9756	95.18
10-1000	0.9800	96.04

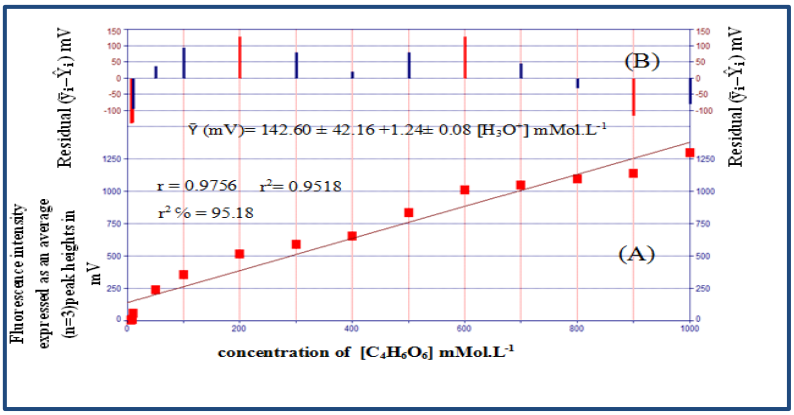

Fig. 3: Calibration graph obtained from Scatter plot for the variation of $C_4H_6O_6$ concentration on: A- Fluorescence intensity, B- residual ($\bar{y}_i$–$\hat{Y}_i$).

4.3.Calibration graph of Citric acid ($C_6H_8O_7$)

A stock solution of citric acid (2Mol.L^{-1}), via (192.12g) in distilled water brings him to a series of citric acid solutions ranging from (5 to 2000) mMol.L^{-1} and injected at the established optimum condition(c.f.section3). An improvement of linearity of ≈ 3% was obtained for concentration range 50-1800 mMol.L^{-1}. This difference can accommodate the use of 5-2000 mMol.L^{-1} concentration range with no noticeable difference in sensitivity or loss of linearity.Table no.5 show Scatter plot of measured data for citric acid,while table no.6 show all range that was used. Table no.(7)show paired t-test between two means ranges(mV) for two acids(Ascorbic and Citric acid).The obtained results indicate that there were no significant differences between two means ranges at 95%confidence interval. Therefore can use one calibration graph for two acids.Figure no.4.show calibration graph for citric acid while a sample of fluorescence intensity vs time profile can be seen in figure no.(5).

Table 5: Scatter plot data for citric acid (i.e.; all measured data were used)

Concentration of $C_6H_8O_7$ in distilled water (mMol.L^{-1})	$\bar{y}_i$ mV	S.D σ_{n-1}	RSD %	$\bar{y}_i$ ± t SEM
5	40	0	0	40 ± 0
7	60	0	0	60 ± 0
9	80	0	0	90 ± 0
10	100	0	0	100 ± 0
50	240	0	0	240 ± 0
100	420	0	0	420 ± 0
200	675	5	0.74	675 ± 12.42
300	840	0	0	840 ± 0
400	915	5	0.55	915 ± 12.42
500	1017	5.77	0.57	1017 ± 14.33
600	1070	10	0.93	1070 ± 24.84
700	1195	5	0.42	1195 ± 12.42
800	1255	5	0.4	1255 ± 12.42
900	1340	0	0	1340 ± 0
1000	1540	0	0	1540 ± 0
1200	1695	5	0.29	1695 ± 12.42
1500	1920	0	0	1920 ± 0
1800	2220	0	0	2220 ± 0
2000	2280	0	0	2280 ± 0

SEM $=\sigma_{n-1}/\sqrt{n}$ Confidence interval of the mean at 95 for n-1$=\bar{y}_i \pm t$ SEM

Table 6: Range of the used concentration for Citric acid

Range of used concentration (mMol.L^{-1})	Correlation coefficient (r)	r^2 %
5-2000	0.9745	94.96
10-1800	0.9802	96.07
50-1800	0.9862	97.25

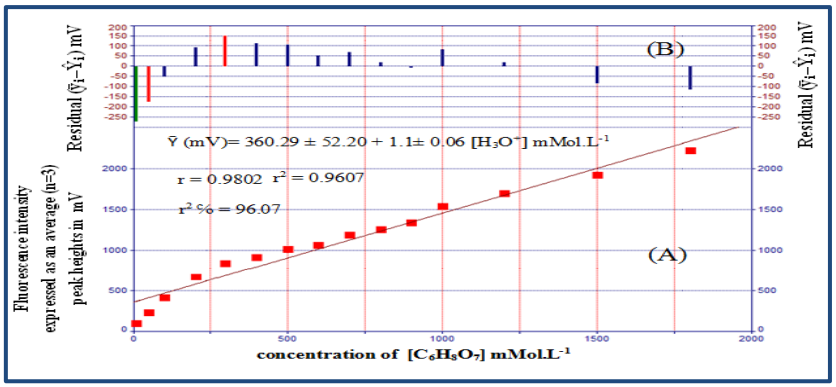

Fig. 4: Calibration graph obtained from Scatter plot for the variation of $C_6H_8O_7$ concentration on: A- Fluorescence intensity, B- residual ($\bar{y}_i$–$\hat{Y}_i$).

Table 7: Paired t-test between two means ranges (mV)for two acids (Ascorbic and Citric acid)

Concentration of two acids mMol.L^{-1}	$C_6H_8O_6$	$C_6H_8O_7$
5	10	40
7	20	60
9	30	80
10	40	100
50	200	240
100	340	420
200	418	675
300	715	840
400	777	915
500	800	1017
600	897	1070
700	960	1195
800	1035	1255
900	1075	1340
1000	1233	1540
1200	2240	1695
1600	2580	1920
1800	2625	2220
2000	2655	2280
df	18	
t_{cal}	0.200	
t_{tab}	2.101	
Sig.(2tailed)	0.843	
$\overline{Xd}$	13.26	
σ_{n-1}	288.33	

$t_{tab} > t_{cal}$ (2.101 > 0. 200) $\overline{Xd}$: Mean for difference between two means ranges, df: a degree of freedom.

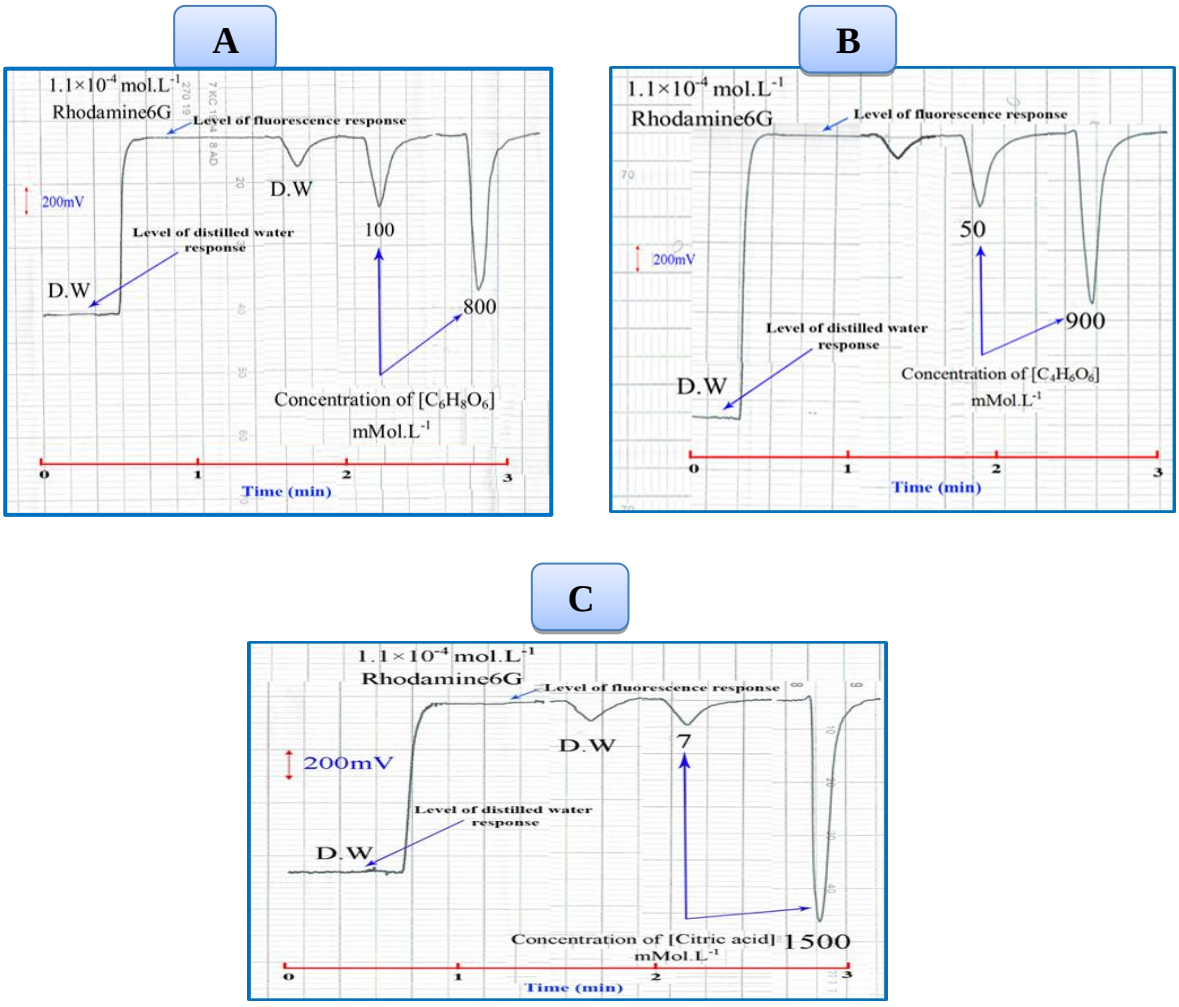

Fig. 5: Effect of variation of [H3O$^+$] concentration mMol.L^{-1}(A-$C_6H_8O_6$, B-$C_4H_6O_6$and C-$C_6H_8O_7$) on response time profile

4.4. Repeatability of weak acids

Five successive injected samples measurements were carried out for repeatability study for the determination of three weak acids [$C_6H_8O_6$,$C_4H_6O_6$ and$C_6H_8O_7$] respectively was 300,400and 500 mMol.L^{-1} via measurements of the Fluorescence intensity quenching. Table no.(8) show the results obtained, while Figure no.(6) shows a kind of quenching of fluorescence -time profile for the used concentrations.

Table 8: Repeatability of acids determined via the quenching of fluorescence

Type of acids	No. of injection to acid 300,400,500mMol.L^{-1}	Fluorescence intensity quenching expressed as peak height (mV)	Average $\bar{y}_i$ mV	Standard deviation S.D	Repeatability RSD%	confidence interval of the mean $\bar{y}_i \pm$ t SEM
Ascorbic acid ($C_6H_8O_6$)	5	720,700, 700,720,700	708	10.95	1.55	708 ± 13.59
Tartaric acid ($C_4H_6O_6$)	5	900,900,900, 900,920	904	8.94	0.99	904 ± 11.10
Citric acid ($C_6H_8O_7$)	5	1000,980,1000,1000,980	992	10.95	1.10	992 ± 13.59

SEM=$\sigma_{n-1/\sqrt{n}}$

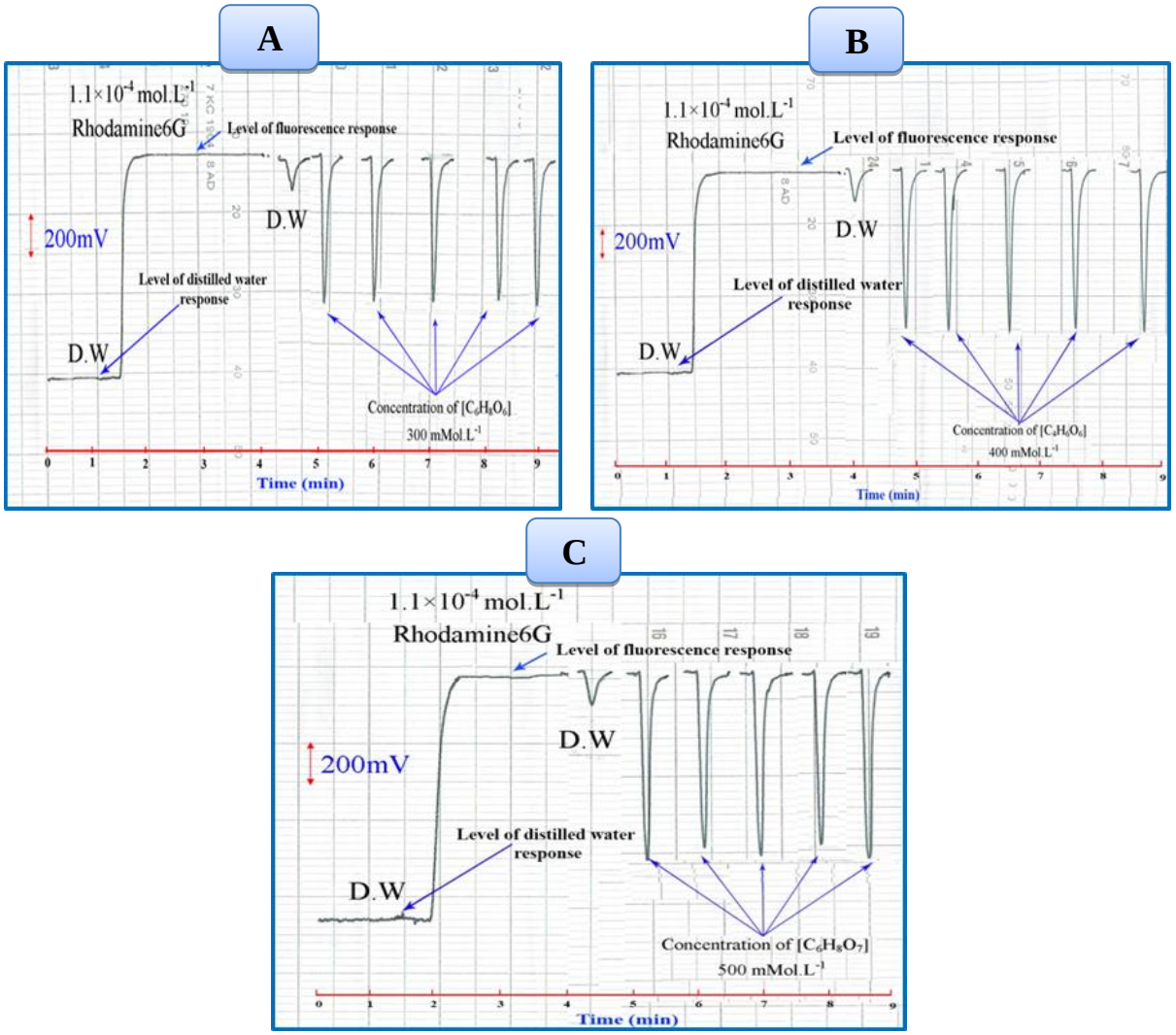

Fig. 6: A Profile of successive repeatability measurements of acids (A:$C_6H_8O_6$, B: $C_4H_6O_6$, C:$C_6H_8O_7$: using532nm solid state laser diode

4.5.Limit of detection for weak acids

The limit of detection of (H_3O^+) was determined using the gradual dilution of the lowest concentration of the analyte in the calibration graph and based on the value of slopeobtained from the linear regression plot as tabulated in table no.(9).Figure no.(7) Show profile of detection of limit for tartaric acid as a sample of measurements.

Table 9: Limit of detection for acids at optimum condition

Type of acid	K_a for acids	Molecular weight of acids g .Mol^{-1}	minimum concentration ($mMol.L^{-1}$)	Practically based on the gradual dilution for the minimum concentration	Theoretical based on the volume of slope X= $3S_B$ / slope
Ascorbic acid ($C_6H_8O_6$)	7.9×10^{-5}	176.12	4	323.356µg/sample	385.276µg/sample
Tartaric acid ($C_4H_6O_6$)	9.2×10^{-4}	150.087	5	344.450µg/sample	373.339µg/sample
Citric acid ($C_6H_8O_7$)	8.4×10^{-4}	192.12	4	352.732µg/sample	538.718µg/sample

S_B: standard deviation of blank solution. , X= value of L.O.D based on slope. L.O.D = limit of detection.

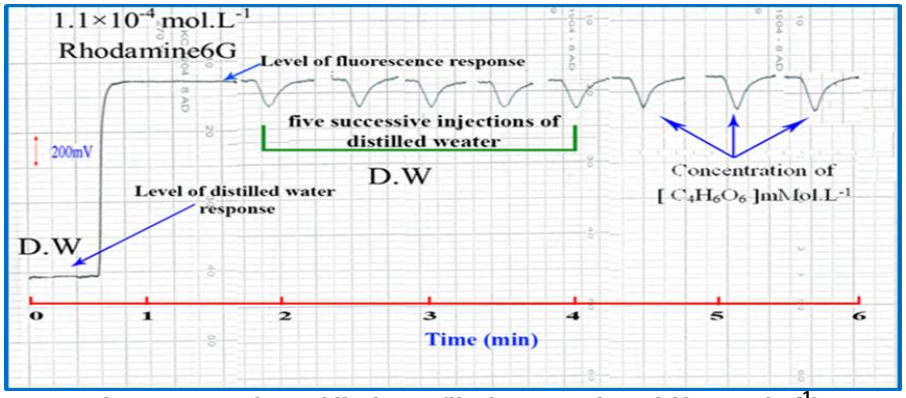

Fig. 7: Detection of limit profile for tartaric acid(5mMol.L^{-1})

4.6.Application of organic and weak acids

The established method was used for the determination of ascorbic acid (Vitamin C) in Vitamin C tablets sample 500 mg Basic Nutrition (UK) using 532 nm solid state laser diode. The standard addition method was applied by preparing a series of solution from pharmaceutical drug via transferring 0.5mL (500mMol.L^{-1}) to five volumetric flask 25mL,followed by addition of 0,0.5,1,1.5,2mL from 500mMol.L^{-1} standard addition of ascorbic acid in order to have the concentration range from 0-40mMol.L^{-1}. Using a classical method (UV-Visible spectrophotometer) a series of solution were prepared of pharmaceutical drug 1mMol.L^{-1} by transferring 0.25 mL to each five volumetric flask 25ml followed by addition of gradual volumes of standard ascorbic acid 0,0.25,0.5,0.75,1mL of (1mMol.L^{-1}). Figure no.8 show the straight–line graph from 0-40 mMol.L^{-1} by new method and 0-0.04 mMol.L^{-1} by classical method. The results were mathematically treated for standard addition method and tabulated in table no.(10) using new method and classical method at confidence interval 95%.Table no.11was shown a practical content of active ingredient at 95% confidence level & efficiency of determination in addition to paired t-test which shows a comparison at two difference paths:

First: Individual t-test A comparison between quoted value (500mg) with new method (532nm solid state laser diode) as the shown in table no.11 (column7) by calculated t-values of drug company and comparison with tabulated t-value (4.303).

A hypothesis can be estimated as follow:

Null hypothesis

There is no significant difference between the mean obtained from one source of one company X and quoted value (μ_o)

$$\textbf{i.e.; } H_o : \overline{X} = \mu_o$$

Alternative hypothesis

There is a significant difference between the mean and quoted value (μ_o)

$$\textbf{i.e.; } H_1 : \overline{X} \neq \mu_o$$

It was noticed that were significant difference between quoted value and measured value $t_{calculated} > t_{tabulated}$ (4.303) at confidence level 95% ; Alternative hypothesis will be accepted and will reject the Null hypothesis which indicate that quoted value is different than what was found practically which is about 97% a recovery.

Second

Paired t-test was used in order to compare between developed method using 532nm solid state laser diode with classical method UV-VIS spectrophotometer as shown in table no.12 , the obtained result indicating clearly there was no significant different between two method at 95% confidence level,since the calculation t-value less than t_{tab}(4.303) for the determination of ascorbic acid in pharmaceutical preparation.

Assumption:

$$\textbf{Null hypothesis } H_0 : \mu_{532nm\ solid\ state\ laser\ diode} = \mu_{UV\text{-}Vis}$$

$$\textbf{Against Alternative hypothesis } H_1 : \mu_{532nm\ solid\ state\ laser\ diode} \neq \mu_{UV\text{-}Vis}$$

Null hypothesis will be accepted and will reject the alternative hypothesis.

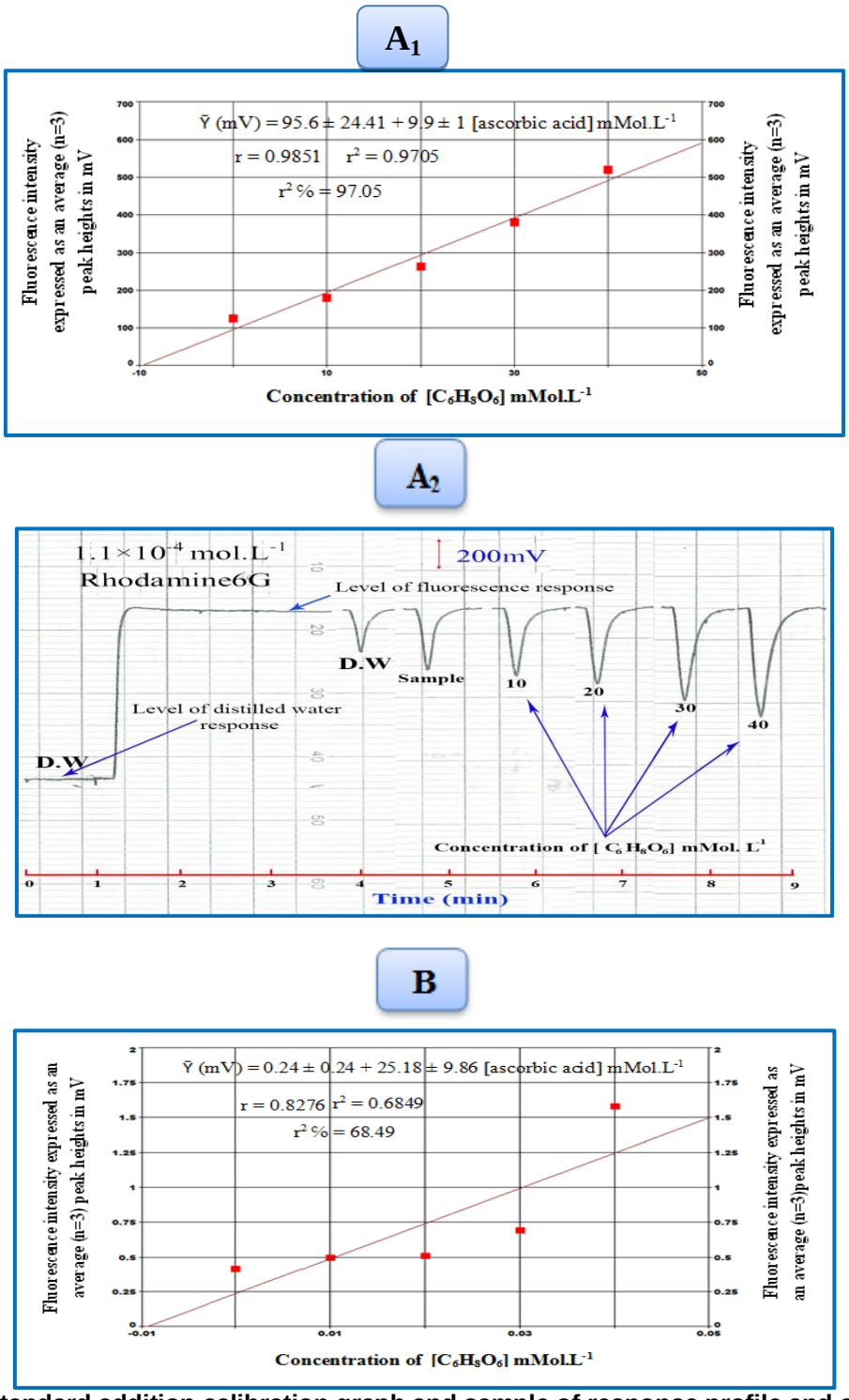

Fig. 8: Standard addition calibration graph and sample of response profile and standard addition calibration plot for the determination of ascorbic acid in pharmaceutical drug A_1, A_2:Vitamin C Basic Nutrition UK using 532nm solid state laser diode,B: Vitamin C Basic Nutrition UK using (UV-Vis spectrophotometer)

Table 10: Standard addition results for the determination of ascorbic acid in pharmaceutical drug using 532 nm solid state laser diode and UV-Visible spectrophotometer

Commercial name ,content company , country,	Sampleweight equivalent to 8.806g (500 mMol.L^{-1}) of the active ingredient (g) using newly method and equivalent to 0.0176g (1mMol.L^{-1})of the active ingredient (g) using classical method	532nm solid state laser diode UV-Visible SP.(classical method for absorbance measurement) $[C_6H_8O_6]$ mMol.L^{-1}					Equation of standard addition curve at 95% for n-2 $\hat{Y}_i$ (mV) = a± s_at+b ±s_bt [X]mMol.L^{-1} $\hat{Y}_i$* = a± s_at+b ±s_bt [X]mMol.L^{-1}	r r^2 r^2 %	Practical concentration mMol.L^{-1} in 25mL and 100mL
		0	10	20	30	40			
		0	0.01	0.02	0.03	0.04			9.657
Vitimine C 500 mg BASIC NUTRITION (UK)	19.0985	125	180	263	380	520	95.6 ± 24.41 + 9.9 ± 1 [X] mMol.L^{-1}	0.9851 0.9705 97.05	482.85
	0.0382	0.421	0.498	0.511	0.692	1.583	0.24 ± 0.24 + 25.18 ± 9.86 [X] mMol.L^{-1}	0.8276 0.6849 68.49	0.00953 0.953

$\hat{Y}_i$= Estimated response in mV for 532nm solid state laser diode, $\hat{Y}_i$*= Estimated value for absorbance , [X] = [ascorbic acid] mMol.L^{-1} , r = Correlation coefficient , Coefficient of determination,r^2% = Linearity percentage

Table 11: Summary of results for paired t-test, practical content and efficiency of determination of ascorbic acid in one sample of pharmaceutical preparation

Confidence interval for the average weight Wi ±1.96 $\sigma_{n-1}/\sqrt{n}$ at 95% (g)	Sample weight equivalent to 8.806g (500 mMol.L^{-1})of the active ingredient Wi (g) using new method and equivalent to 0.0176g (1 mMol.L^{-1}) of the active ingredient Wi (g) using classical method	Theoretical content for the active ingredient at 95% (mg) μ ±1.96 $\sigma_{n-1}/\sqrt{n}$	Practical content of active ingradient		Efficiency of determination (Rec. %)	Individual comparison $\overline{X}$ -μ $\sqrt{n}$ /σ_{n-1} new method and classical method with Quoted value t 0.05/2 ,= 4.303
			In 100ml of samples Wi ±4.303$\sigma_{n-1}/\sqrt{n}$ (mg) for (n=3) ,at 95% (g)	In tablets Wi ±4.303$\sigma_{n-1}/\sqrt{n}$ (mg) for (n=3) ,at 95% (mg)		
		532nm solid state laser diode UV-Visible spectrophotometer				
1.0844 ± 0.00329	19.0985	500 ± 1.5170	8.5040 ± 0.130	482.85 ± 7.381	96.57	I-9.998I> 4.303
	0.0382	500 ± 1.5170	0.0168 ± 0.00025	476.91± 7.097	95.38	I-13.998I> 4.303

n= no.of sample = 3 , $t_{0.025,\infty}$ = 1.96 at 95%, μ: quoted value (500mg) ,$\overline{X}$ = $\overline{W_i}$=Practical content (mg)

Table 12: Paired t-test for comparison between 532 nm solid state laser diode with classical method using standard addition method for determination of ascorbic acid in pharmaceutical preparation

Theoreticalcontent for the activeingredient (mg)	Practically content of vitamin c (mg)	X_d	$\overline{X_d}$	σ_{n-1}	$t_{cal} = \overline{X_d}\sqrt{n}/\overline{\sigma_{n-1}}$	t_{tab} at 95%
500	482.85	-17.15	-20.12	4.518	I- 7.713I< 12.706	
	476.91	-23..09				

Xd : Difference between two methods, $\overline{X_d}$: Difference mean, $\overline{\sigma_{n-1}}$: Difference standard deviation n= no.of methods =2

CONCLUSION

The work presented in this research shows the capability of accepting an alternative method for the analysis and determination of(H_3O^+) ion in organic acidwith good repeatability by 532 nm solid state Laser diode with continuous flow injectionmethod.The methodcharacterized by simplicity, speed and accuracy. The standard addition method was applied for determinationascorbic acid in vitamin C tablets 500mg UK. Statistical analysis for the results using t-test showed significant differencebetween quoted value and measured value there for Alternative hypothesis will be accepted and will reject the Null hypothesis and also showed no significant difference between new method using 532nm solid state laser diode with classical method UV-VIS spectrophotometer in precision,efficiency and accuracy.

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