طريقة للبريق الكيميائي الكلي وتحليل الحقن الجرياني لتقدير الهستادين

عصام محمد علي شاكر، نغم شاكر تركي ،وسام صلال عليوي*
قسم الكيمياء ،كلية العلوم ،جامعة بغداد
***كلية الزراعة ، جامعة بغداد**

الخلاصة

قدر الهستادين بوساطة قياس التألق الكلي الناتج من تفاعل البريق الكيميائي لنظام : لومينال – بيروكسيد الهيدروجين – هيدروكسيد الصوديوم – هستادين الذي يعطي بريقاً أزرق – بنفسجي له قمة انبعاث عند 425 نانوميتر . استخدم جزء من هذا الضوء المتولد مصدرا داخليا لتشعيع جزيئة متفلورة مستقبلة (الفلورسين) لتحضير نظام : فلورسين / لومينال – بيروكسيد الهيدروجين – هيدروكسيد الصوديوم – هستادين ، قيس التألق الكلي (المتبقي من البريق + الفلورة) في خلية زجاجية حلزونية مصممة لهذا الغرض . عولجت البيانات باستخدام معادلتين : الدرجة الاولى ومعادلة الدرجة الثانية وكانت خطية للمدى (0.002–0.005) مول . لتر$^{-1}$ ومعامل ارتباط 0.9879 مع معامل تقدير 97.59% باستخدام معادلة الدرجة الاولى وعند حجم لانموذج محقن 70 مايكروليتر، بينما كان معامل الارتباط للمدى نفسه (0.002–0.05) مول . لتر$^{-1}$ 0.9881 ومعامل التقدير 97063% لمعادلة الدرجة الثانية ، واجراء تحليل للمتباينات لكلا المعادلتين . تم التوصل الى حد كشف 0.5 ملي مول . لتر$^{-1}$ ولوحظ عند اجراء مقارنة في القياس بالبريق الكيميائي أو التألق الكلي انه لا يوجد فرق جوهري بين الطريقتين .

Total luminescence (Chemiluminescence & Fluorescence)-FIA Method For The Determination of DL-Histidine

I . M.A. Shakir , N. S. Turkie, W. S. Ulaiwi
Department of Chemistry, College of Science, University of Baghdad
College of Agriculture, University of Baghdad

Abstract

Histidine was determined via measurement of total luminescence (i:e creation of chemiluminescence and insitu irradiation of released light to an acceptor fluorophore molecule to initiate fluorescence from fluorescien molecule in flat – spiral micro cell designed for this measurement . A detailed description of robust linear equation for the range of 0.002 – 0.05 mol.L^{-1} for a sample size of 70 µL with a correlation coefficient of 0.9879 and a coefficient of determination of 97.59% while for a quadratic model of the same concentration range was 0.9881 correlation coefficient and 97.63% coefficient of determination. Analysis of variance was conducted for both kinds of models . It indicated that their was no significant difference and both equations can be used to represent the data obtained

Introduction

Histidine is an important chemical compound and many methods were developed for its determination which was specified by accuracy and precision. Fluorometric measurement of histidine is based on the formation of a fluorophore with o-phthaladehyde by using condensation reaction in a strongly alkaline medium to form a rather labile fluorescent compound which is stabilized by acidification. The reaction was used to conform histidinemia [1]. A fluorescence detection of histidine at 385 and 265 nm was accomplished after derivatization with 2-hydroxy-1-naphthaldehyde by using HPLC [2]. Capillary zone electrophoresis with pre-column naphthalene-2, 3-dicarboxaldehyde derivatization and fluorescence detection of (3.8×10^{-9})M (S/N=3) [3]. Were used chemiluminescence detection coupled with flow injection analysis using N-Bromosuccinimide with sodium fluoresciene as an acceptor fluorophore agent were used and measurement at 535 nm was also used [4].

This kind of energy transfer was used by different works [5,6]. Many years previous to reference were mentioned above [4] . Also a successive energy transfer was used by using different acceptor fluorophore [7-12]. In the present paper, histidine was determined by measuring the chemiluminescence as well as fluorescence signal simultaneously as total luminescence. Sensitivity was increased as compared to chemiluminescence alone. Rapid assay can be achieved within 30 seconds.

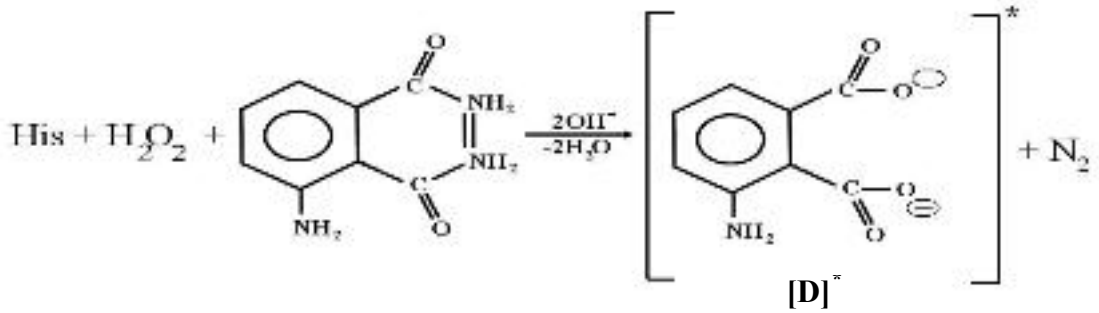
Experimental

Reagents: 0.1M (DL-Histidine) in distilled water, 5-amino phthylhydrazide (1.0 mmol.L^{-1} in 0.2 mol.L^{-1}-NaOH) , 3.0 mol.L^{-1}-H_2O_2 (purified by ion exchange resin), sodium fluoresciene 2.0 mmol.L^{-1}, and sodium hydroxide 0.5 mol.L^{-1} as a carrier stream.

Apparatus: Home set up chemiluminometric measuring unit. (9875QB) PMT, power supply 0.0-1.6 KV, amplifier (Dual detector), x,y-t chart recorder, perstaltic pump (3-channel), 0.5, 1.0mm (I.D) silicone or tygon tube, figure -1- shows the detail of instrument setup using a spiral flat flow cell of 100µL size.

Methodology

A sample of 70μL of 0.1M (DL.Histidine) solution was injected via six port injection valve into the carrier stream (0.1 mol.L^{-1}-NaOH) line (as shown in Fig.1) repeatidly for the total luminescence measurement at flow rate of 1.5 mL.min^{-1}. While the other two lines (0.5 mol.L^{-1} H_2O_2) at 2 mL.min^{-1} and 0.5 mmol.L^{-1}-Luminol which contain 0.5 mmol.L^{-1}-Sodium fluoresciene and 0.2 mol.L^{-1}- NaOH at 2mL.min^{-1}. Both lines were met before reaching the flow cell where they met and mix at the flow cell via the cells two inlets. The signal obtained is measured as current(whether pA or nA or even μA or via chart recorder measuring mV against time). Only single peak was obtained which represents the total luminescence resulted from chemiluminescence and fluorescence obtained by internal irradiation of chemiluminescence photon released by breakdown of luminol molecules. Actually it is believed that histidine molecule enhances the chemiluminescence of luminol molecule oxidation by the hydrogen peroxide most probably as follows:



$[D]^* \rightarrow [D] + h\upsilon_1$ (chemiluminescence step)

$D + h\upsilon_1 + \text{sodium fluoresciene} \rightarrow D + h\upsilon_1 + [\text{sodium fluoresciene}]^*$

remaining

$[\text{sodium fluoresciene}]^* \rightarrow \text{sodium fluoresciene} + h\upsilon_2$

green

$h\upsilon_1 + h\upsilon_2 = \text{total luminescence}$

remaining fluoresciene

Effect of Fluoresciene Concentration on the Total Luminescence:

Series of solutions of variable concentrations of fluoresciene extended from 0.05-1.00 mmol.L^{-1} were added to a constant concentration of luminol (0.5mmol.L^{-1}) for three variable concentrations of histidine (0.01, 0.02, 0.03) mol.L^{-1}. Figure 2 shows the three curves obtained for total luminescence vs concentration of added fluoresciene on luminol solutions. The results indicate that the most suitable concentration of fluoresciene is 0.5 mmol.L^{-1}. At lower concentrations of fluoresciene the exited amount of this reagent is not enough for the reception of energy released by chemiluminescence reaction while at higher concentration of fluoresciene self quenching and inner filter effect play an important role in decreasing ,the total luminescence (i:e remaining chemiluminescence plus simulated fluorescience).

Scatter Plot (Variation of Total Luminescence vs concentration of DL-Histidine)

A series of DL-Histidine solutions were prepared ranging from 0.00-0.06 mol.L^{-1}. (viz 0.002, 0.005, 0.008, 0.010, 0.015, 0.020, 0.025, 0.030, 0.040, 0.050, 0.055 and 0.060 mol.L^{-1}). Total luminescence was measured for 70μL samples injected successively for three times. Average, standard deviation and confidence interval of the average values in addition to the estimated values for the range 0.002-0.050 mol.L^{-1} using linear regression and quadratic equation are tabulated in table 1.

Table 2 (a,b) gives the detailed treatment for regression analyses whether linear or quadratic. Table 3 (a,b) tabulates all the analyses of variance for both equations. It can be concluded that there were no significance difference between the two equations (indicated by

the range used, correlation coefficient and coefficient of determination). The linear regression equation is more representative as it can be seen from table 3 (a,b) that the F-value from linear plot is 325.08 while that for the quadratic plot was 143.93. And this indicates that less residual values are obtained with more precision, figure 3(a,b) shows the linear regression and quadratic regression plots.

Limit of Detection

Successive dilutions approach of lowest concentration that appear in linear regression plot was used for determination of detection limit in which a clear signal above distilled water at (S/N=3) was obtained for thirteen measurements from 0.0005 mol.L^{-1}. Therefore, (0.5 mmol.L^{-1}) is regarded as a limit of detection. Figure 4 shows the kind of response that was obtained for such concentration. Comparison between chemiluminescence and total luminescence method for the assay of DL-Histidine was made. Total luminescence was compared with chemiluminescence. Figure 5 shows the plot of total luminescence -response against luminescence response with a correlation coefficient of (0.9957) and regression equation total luminescence (nA)=1.12 + 1.02 [Chemiluminescence] nA respectively, "which indicate unbiased measurements for both methods in the range used".

Discussion

The work presents a sensitive, less time consuming method for the determination of DL-Histidine. High limit of detection was obtained (0.5mmol.L^{-1}) at a sample size of 70µL. The method is repeatable with a good repeatability of 1.09% for 0.04 mol.L^{-1}. Comparison with chemiluminescence method shows no bias for either method but this does not necessarily apply for all amino acids as work is in progress for other amino acids. For DL-Histidine no preference was made for either method except for limit of detection. The comparison was conducted by using the same instrumental setting; therefore the comparison was meant for the chemistry involved and not instrumentation involved.

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Table 1: Summary of linear regression and quadratic estimated values

[Histidine] mol.L^{-1}	Average response obtained for n=3 $\bar{y}_i$ (nA)	Standard deviation σ_{n-1}	Precision R.S.D%	Confidence interral of the average at 95% confidence	Estimated values (L.R.E) $\hat{y_i} = a + bx$ $\hat{y_i}(nA)$	Estimated value (Q.R.E) $\hat{y_i} = a + bx + cx$ (nA)
0.002	2.716	0.02	0.736	2.716±0.049	1.594	1.738
0.005	3.319	0.04	1.205	3.319±0.099	2.827	2.907
0.008	3.921	0.05	1.275	3.921±0.124	4.061	4.085
0.010	4.349	0.06	1.379	4.349±0.149	4.884	4.875
0.015	5.210	0.07	1.344	5.210±0.174	6.940	6.868
0.020	8.061	0.09	1.112	8.061±0.224	8.996	8.887
0.025	11.144	0.13	1.167	11.144±0.323	11.053	10.929
0.030	14.430	0.19	1.317	14.430±0.472	13.109	12.998
0.040	18.340	0.20	1.091	18.340±0.496	17.222	17.209
0.050	20.530	0.25	1.218	20.530±0.621	21.334	21.522

L.R.E.: Linear Regression Equation
Q.R.E:Quadratic Regression Equation

Table (2a): Summary of linear regression data analysis

Measured [Histidine] mol.L^{-1}	Concentration range (mol.L^{-1}) for n=10	Linear regression equation at 95% confidence $Y_1\hat{}$=a±S_at+b±$S_b$tx	r r^2%	$t_{tab.}$ L_{n-2}	$t_{cal.}$ $t = \frac{\lvert r \rvert \sqrt{n-2}}{\sqrt{1-r^2}}$	S_{Er}=$\frac{1-r^2}{\sqrt{n}}$	r±S_{Er}
0.00-0.05	0.002-0.05	0.77±1.07+411.27±42.43x	0.9879 97.59%	1.86<<18.03		0.0076	0.9879± 0.0076

$y\hat{}$ = CL.I (av.Pk.ght)n = 3)nA
x = [Histidine] mol.L^{-1}
S_{Er} = Standard Error of the Correlation Coefficien

Table (2b): Summary of quadratic regression data analysis

Measured [Histidine] $mol.L^{-1}$	Concentration range ($mol.L^{-1}$) for n=10	Quadratic regression equation at 95% confidence $\hat{Y}_2 = a \pm S_a t + b \pm S_b tx \pm c \pm S_c tx^2$	r r^2, %	$t_{tab.}$ L_n-2	$t_{cal.}$ $t = \frac{\lvert r \rvert \sqrt{n-2}}{\sqrt{1-r^2}}$	$S_{Er} = \frac{1-r^2}{\sqrt{n}}$	$r \pm S_{Er}$
0.00-0.05	0.002-0.05	$0.96 \pm 1.69 + 386.10 \pm 169.72x + 500.85 \pm 325.67x^2$	0.9881 97.63%	1.86<<18.15		0.0075	0.9881 ± 0.0075

Table (3a): Analysis of variance for linear regression equation

Source	Sum of square Ssq	Df	Mean squares Msq	F-statistic= $\frac{S_1^2}{S_0^2}$
Regr(due to regression) Error (about regression) Total	378.96 9.33 388.29	1 n-2=8 n-1=9	S_1^2=378.96 S_0^2 = 1.17	325.08

Table (3b): Analysis of variance for quadratic regression equation

Source	Sum of squares Ssq	Df	Mean squares Msq	F-statistic= $\frac{S_1^2}{S_0^2}$
Regr(due to regression) Error (about regression) Total	379.07 9.22 388.29	2 n-3=7 n-1=9	S_1^2=189.53 S_0^2 = 1.32	143.93

Df = Degree of freedom

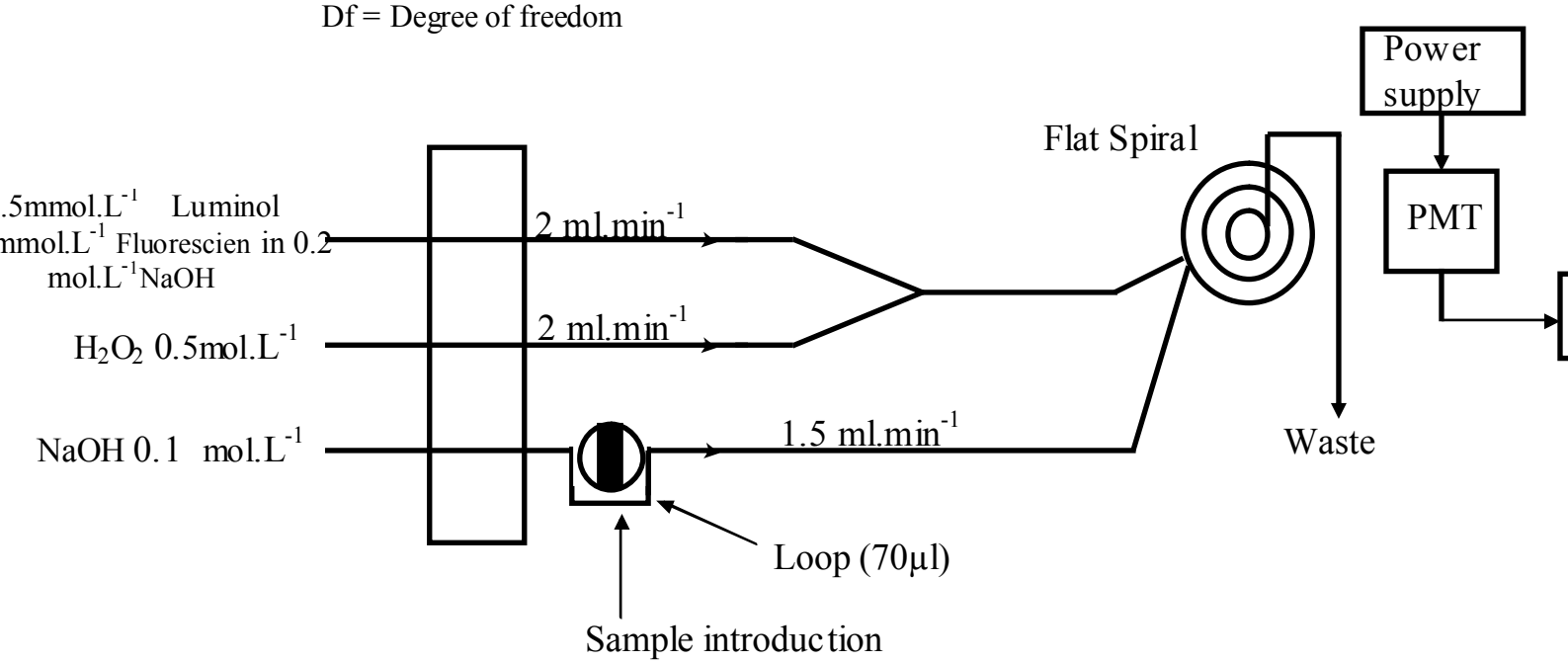


Fig.(1): Schematic flowgram of the total luminescence setup for the determination of DL-H
Luminol/ Fluorescien –OH^--H_2O_2-Histidine

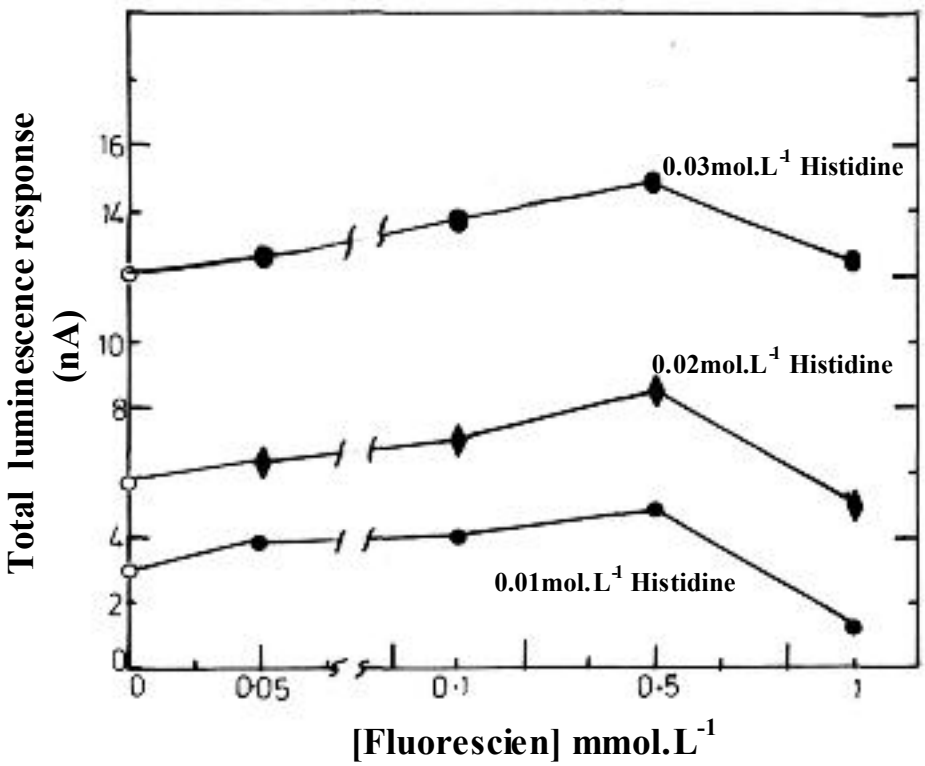
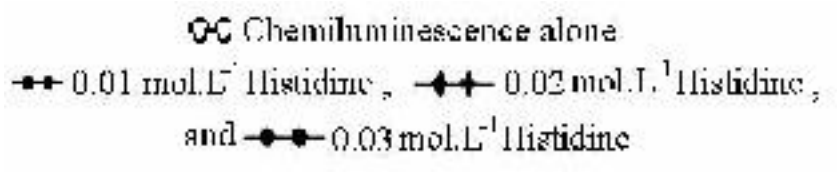


Fig.(2): Variation of total luminescence expressed in nA response versus fluorescien concentration in mmol.L^{-1} for luminol/ fluorescien-H_2O_2-OH^-Histidine



Note :(Zero concentration of fluorescien indicater it is only luminol with no added fluorescien)

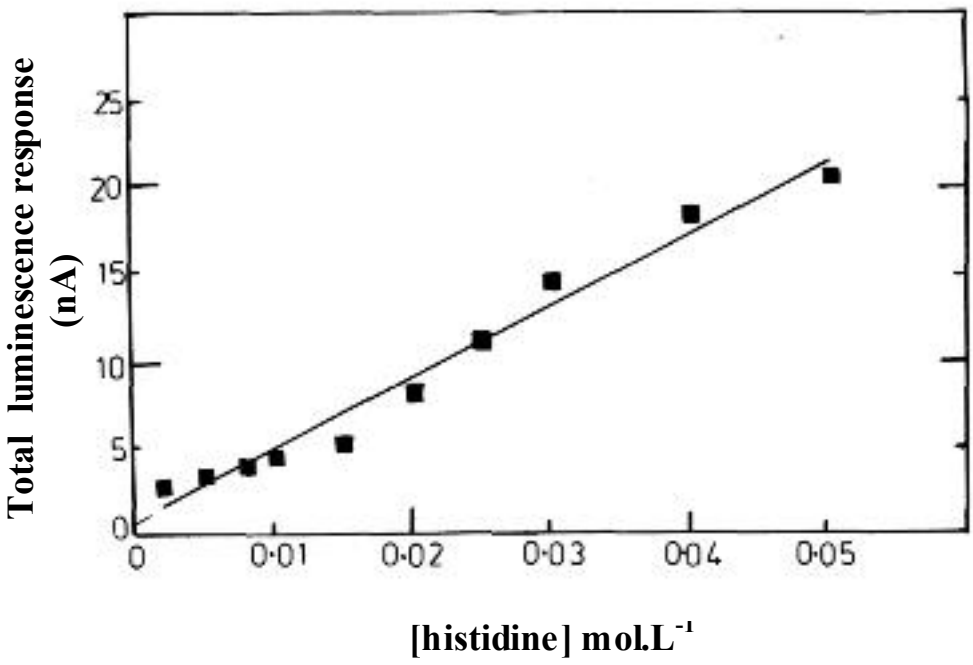


Fig.(3a): Variation of total luminescence versus Histidine concentration (Scatter plot) and the liner regression plot (indicated by solid line)

Fig.(3b):Variation of total luminescence versus Histidine concentration (Scatter plot) and the quadratic regression plot (indicated by solid line)

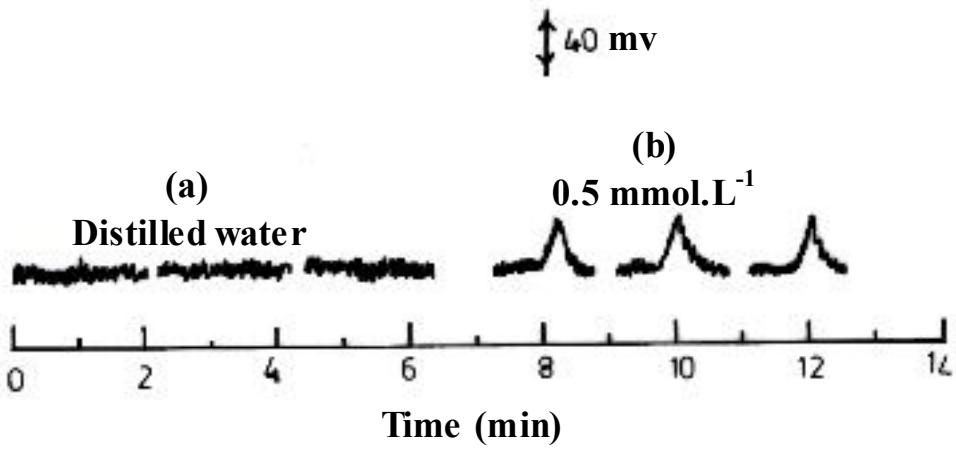


Fig. (4):Distilled water signal (a) and high level of detection of DL- Histidine (b)

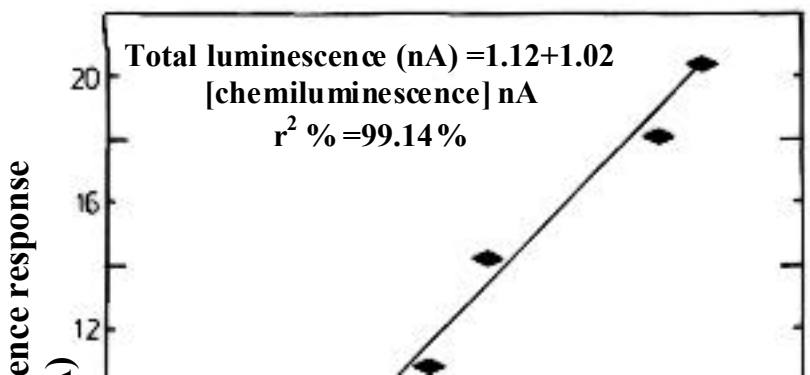


Fig.(5): Comparison between total luminescence method and chemiluminescence for the assay of Histidine