Novel Chemiluminometric-FIA Method for The Determination of DL-Histidine Via Loaded Ion Exchange Resin with Cobalt(II) Ion

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Abstract

This research work presents completely new approach for the determination of DL-Histidine in a sensitive easy method. It relies on the use of cation exchange resin loaded with Co(II) ion. DL-Histidine replaces Co(II) ion entrapped in the resin bed structure giving to a release of Co(II) ion. The cobalt (II) ion catalyses the peroxide decomposition leading to a chemiluminescence reaction. The energy of emitted photons is measured and related to the concentration in a single well defined profile. The linearity for the 0.0-1000 μmol.L^{-1} has correlation coefficient 0.9935, and a coefficient of determination 98.71% with a limit of detection of 50 nmol.L^{-1} using 25μL sample. While using quadratic regression gave a correlation coefficient for 0.0-1000 μmol.L^{-1} of 0.9981 with a coefficient of determination 99.63%. further treatment of data were handled and ANOVA for both equations were conducted which proves that both equations can be used for the range given above. Also a chromatographic calculation approach was conducted for assumed theoretical plates and HETP. Also H_{ex} was calculated at approximatly D_m = 1. as an approximation. The method is at further detailed study.

Introduction

Histidine is part of the digestive enzyme chymotrypsin[1]. It's action by two stages. The first stage it act as an alcohol by breaking the peptide chain. The products are an amine-the liberated portion of the substrate molecule. While in the second stage the enzyme ester is hydrolysed, which yield a carboxylic acid.

The histidine and protonated histidine act a general base and acid in two successive nucleophilic substitution reactions. Histidine is an essential amino acid, has a positively charged imidazole functional group. It is regarded as rare amino acid most notably for the aromatic imidazole ring in its side chain.

Figure no.1 shows the dissociation curve of histidine[2] as all amino acids have at least two ionizable groups. And their net charge therefore depends on the pH value. The COOH groups at the α-carbon atom have pKa values of between 1.8 and 2.8 and are therefore more acidic than simple monocarboxylic acids. The basicity of the α-amino function also varies, with pKa values of between 8.8 and 10.6, depending on the amino acid. Acidic and basic amino acids have additional ionizable group in their side chain. Details of dissociation are shown in Figure no.1.

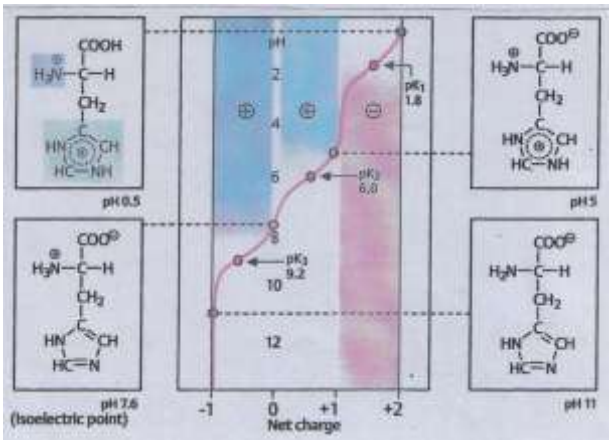

Figure (1): dissociation curve of histidine

It was reported[3] that histidine was determined colorimetrically by the reaction of iminazoles with diazobenzene sulphonic acid which was introduced into protein chemistry and was used for on approximate quantitative estimation of histidine[3]. Also a rapid HPLC method for the determination of histidine in body fluid was developed. The method was based on the separation by a reversed-phase, ion-pair chromatography followed by selective post column detection of histidine with fluorescence derivatization using orthophthalaldehyde[4]. Ion exchange chromatography of free amino acids in aqueous fluid and lens of the human eye were studied[5].

Histidine-tagged proteins sensor was developed[6] for direct detection and identification.

A gas chromatographic - electron-impact mass spectrometric method[7] was used for the determination of stable isotopically substituted histidine in human plasma. It was derivatized to alpha N-trifluoroacetyl-imN-carbethoxy-histidine n-butyl ester (TCB) derivative by a three-step reaction.

A procedure was developed[8] for accurate measurements of histidine decarboxylase in tissues expressing low level of enzymatic activity.

Capillary zone electrophoresis[9] with pre-column naphthalene - 2,3-dicarboxaldehyde derivatization and fluorescence detection 7.8×10^{-9}M was reported as detection limit at ((S/N)=3).

High-performance capillary electrophoresis[10] on a fused silica column was used for determination of histidine in human skin. Histidine was determined[11] as part of many amino acids used for comparison method of derivatization with 2-hydroxy-1-naphthaldehyde, forty minutes is required for each sample. A potentiometric method based on carbon paste electrode modified[12] with tetra-3,4-pyridinoporphirazinato copper(II) for L-histidine determination in linear response of 2.4×10^{-5} - 1.0×10^{-2} M. The detection limit was 2×10^{-5} M, histidine was selectively enhances the reaction of luminol with Mn(II) salts in a basic medium[13]. Enhanced electrogenerated chemiluminescence of luminol using flow injection technique was used for determination of histidine[14] at a detection limit of (S/N=3) of 0.56 μMol.L^{-1}.

Histidine oxidase was immobilized on tresylated poly (vinyl alcohol) beads where H_2O_2 is released and determined[15]. Chemilumino-metrically by flow through sensor containing the immobilized peroxidase detection limit of 0.01 mmol.L^{-1} (S/N=3) was reported.

In this piece of research work, histidine molecules substituted by cation exchange resin previously loaded with Co(II) ion. Released Co(II) ion will behave as a catalyst for the decomposition of H_2O_2 which attack the luminol molecule releasing blue chemiluminescence emission which is recorded and related to concentration of histidine.

Experimental

Reagents:

DL-Histidine (BDH) (0.1 mol.L^{-1}): 100 mL was prepared. Luminol (1 m.mol.L^{-1}) in 0.2 mol.L^{-1} KOH was prepared. Purified H_2O_2 (Via removal cations through the use of cation exchange resin (Amberlite-120. H-form and passing through anion exchange resin. Amberlite-400. OH-Form for removal of anions) 0.5 mol.L^{-1}. potassium hydroxide 0.5 mol.L^{-1} was prepared, Amberlite-120 H-form cation exchange resin used in a column of 3 mm (I.D.). 1m length (Teflon tube).

Apparatus:

Home-made build up system for the measurement of low intensity photons generated via chemiluminescence Dual channel optometer (United detector technology U.S.A), Dc-power supply 0-1.6 kV (JOBIN YVON (France)), PMT (9875QB) x & y-t chart recorder CI032. Kompensograph-siemens (Germany).

Procedure:

Figure no.2 shows the flowgram used for the determination of histidine. The ion exchange column should be loaded by Co(II) ion previous to work and washed well until negative response obtained by luminol test (i.e no released Co(II) ion from resin) followed by continuous fed of distilled water to avoid any released Co(II) ion until steady signal free of cobalt is obtained. A sample of 25μL was injected via six port Teflon made injection valve into the manifold system where it takes just a little more than 4 minutes for the passage and exchange of histidine molecule with binded cobalt(II) ion on resin beads surface. Released Co(II) ion migrate through the column due to the flow of a carrier stream. At the end of the column cobalt (II) ion meets luminol-H_2O_2 at the flow cell inlet where released photons is detected and measured via UV-Vis-NIR PMT. The signal is processed to read either in PA or nA, μA digitally or the response is recorded on x & y-t chart recorder at variable ranges based on the signal obtained.

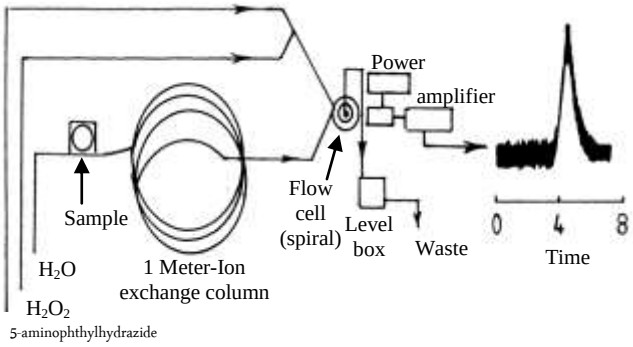

Figure (2): Schematic flow gram for the determination of DL-Histidine using resin bed charged with Co(II) ion.

Variation of Chemluminescence Response vs. Histidine Concentration

Series of variable (0, 10, 20, 40, 60, 80, 200, 400, 600, 800, 1000 μmol.L^{-1}) concentration of DL-Histidine from a stock solution of 100 mmol.L^{-1} were prepared after a successive preliminary experiment for the choise of the working range. A sample of 25μL was introduced each time for every single measurement. For each concentration three measurements were taken. Table no.1 tabulated all the data obtained i.e; y_i, σ_{n-1}, %R.S.D., Confidence interval at $\alpha_{0.05}$, $\hat{y_i}$, and the residual values for both equations; linear regression and quadratic equation while Figure(3) shows the scatter plot and the linear regression plot (solid line). While the quadratic regression plot. (dotted line). Figure (4) shows residual plot vs. histidine concentration for simple linear regression equation while Figure (5) shows residual plot vs. histidine concentration using quadratic equation of the form of $y=a+bx+cx^2$. Table no.2-A,B tabulate the values of correlation coefficient of linear and quadratic

analysis. It include the values of constant a, b and c. for the two equation with the confidence interval at $\propto_{0.05}$ probability. Table no.3-A, tabulate all the treatment of data obtained with up to date manipulation for linear regression plot. Tables no.3-B shows the quadratic plot treatment (Analysis of variance)[16-18]. Figure (6) shows the profile of registered responses.

Table.1: Summary for variation of histidine concentration (μmol.L^{-1}) against chemiluminescence expressed in nanoampere for the system, Histidine ↔ Co(II)-Luminol-H_2O_2

[Histidine] μmol.L^{-1}	av.pk.hgt. (n=3) $\overline{y_i}$	σ_{n-1}	Repeatability R.S.D.%	$\overline{y_i} \pm t_{0.05}\frac{\sigma_{n-1}}{\sqrt{n}}$	LR		QE	
					Predicted $\hat{y_i}$	Residuals $\overline{y_i} - \hat{y_i}$	Predicted $\hat{y_i}$	Residuals $\overline{y_i} - \hat{y_i}$
0	0.0	0.00	0.00	0	104.48	-104.48	33.12	-33.12
10	11.8	0.23	1.95	11.8±0.57	131.68	-119.88	70.12	-58.32
20	64.8	0.35	0.54	64.8±0.87	158.87	-94.07	106.92	-42.11
40	163.3	0.57	0.35	163.3±1.42	213.26	-49.96	179.83	-16.53
60	285.3	0.98	0.34	285.3±2.43	267.65	17.65	251.88	33.42
80	337.3	1.35	0.40	337.3±3.35	322.04	15.26	323.06	14.24
100	453.3	2.33	0.51	453.3±5.79	376.43	76.87	393.36	59.94
200	823.0	4.57	0.56	823.0±11.35	648.39	174.61	731.75	91.25
400	1386.3	3.32	0.24	1386.3±8.25	1192.29	194.01	1342.98	43.32
600	1763.3	4.39	0.25	1763.3±10.90	1736.19	27.11	1866.82	-103.52
800	2263.0	2.38	0.11	2263.0±5.91	2280.09	-17.09	2303.26	-40.26
1000	2704.0	3.98	0.15	2704.0±9.89	2824.00	-120.00	2652.31	51.69

av.pk.hgt: average peak height
LR : linear regression
Q.E: Quadratic Equation

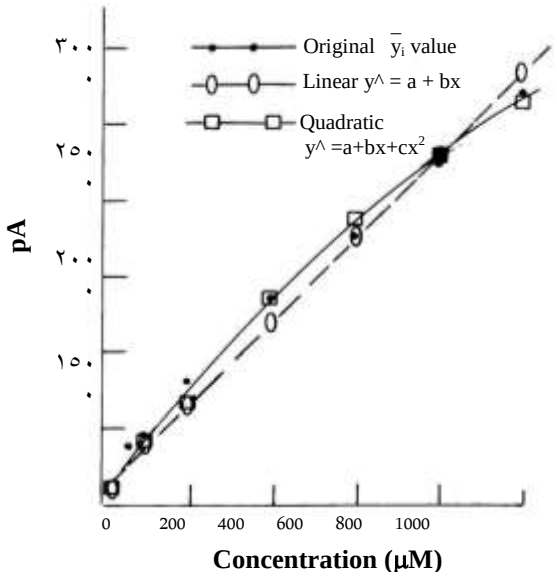

Figure (3): Scatter diagram of chemiluminescence vs. concentration of DL-Histidine (⟷), linear regression plot of y^=a+bx (- -), and quadratic plot of y^=a+bx+cx^2 (—).

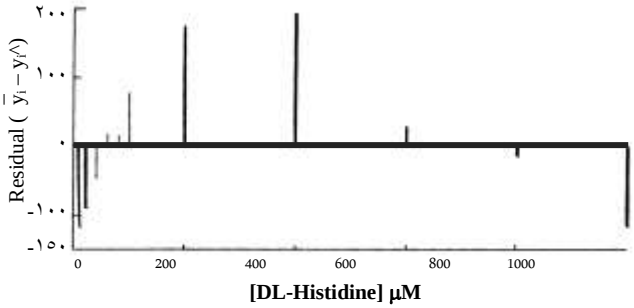

Figure (4): Residual ($\bar{y}_i - y_i^{\wedge}$) vs. concentration of DL-Histidine using linear regression of robust linear equation of the form of y=a+bx

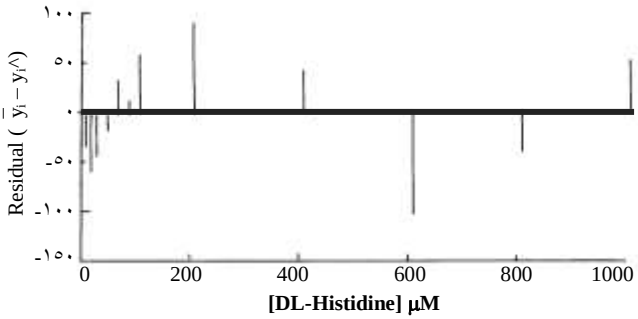

Figure (5): Residual values vs. concentration of DL-Histidine using Quadratic regression analysis

Table.2. Summary of chemiluminescence vs. histidine concentration expressed by linear regression treatment and quadratic regression treatment for the 0-1000 µmol.L^{-1} concentration range at 95% confidence for DL-Histidine

Concentration range for n=12 (µmol. L^{-1})		**Equations at 95% confidence interval for(n-2)**	**r r^2%**	**$t_{cal.}$** $t=\frac{\lvert r\rvert\sqrt{n-2}}{\sqrt{1-r^2}}$	**$t_{0.05,\ n-2}$**
A	0-1000	104.48±76.59 +2.72±0.18[x]	0.9935 98.71%	27.65 >> 1.81	
B	0-1000	33.12±51.42+3.71 ±0.39[x]-0.001 ±0.0004[x]^2	0.9981 99.63%	51.62 >> 1.81	

A= linear regression: chemiluminescence (nA)=a±S_at+b±S_bt[DL-Histidine]µmol.L^{-1}
B= Quadratic regression: chemiluminescence
(nA)= a±S_at+b±S_bt[DL-Histidine]µmol.L^{-1} + c±S_ct[DL-Histidine]^{2}µmol^2.L^{-2}
[x] = [Histidine]µmol.L^{-1}

Table.3.A. Analysis of variance for linear plot

Source	**Sum of square (SSq)**	**D_F**	**Mean square (Msq)**	**(Cal. F) F-Statistic**	**(tabulated F) F^1_{10}**
Due to regression	9681743.30	$\nu_1 = 1$	9681743.300	746.49 >> 4.96	
About regression (error)	126642.96	$\nu_2 = 10$	12664.296		
Total	9808386.30	11			

Table.3.B. Analysis of variance of quadratic plot data

Source	**(SSq)**	**D_F**	**(Msq)**	**(Cal. F) F-Statistic**	**(tabulated F) F^2_9**
Due to regression	9771715.20	2	4885857.60	1199.11 >> 4.26	
About regression (error)	36671.09	9	4074.57		
Total	9808386.30	11			

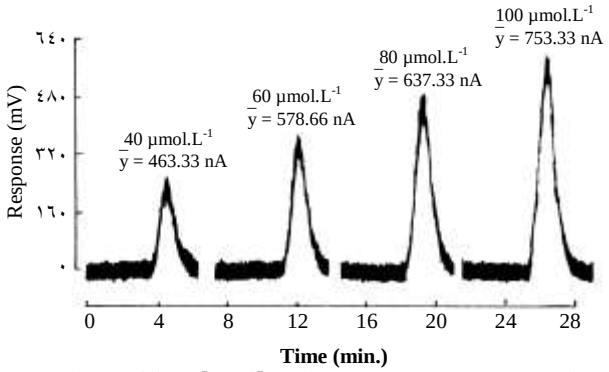

Figure (6): Chemiluminescence response vs. time for variable concentration of DL-Histidine; via the exchange of Histidine molecule with entrapped Co(II) ion on amberlite resin. Millivolt response as nano ampere are shown together on the real chart

Limit of detection

Thirteen successive measurement of 50 nmol.L^{-1} were conducted above background a sample response is shown in Fig.(7). which enlarged three times. With an $\bar{y}$ =274.1 nA, σ_{n-1} = 14.2 and R.S.D.% of 5.2% for (n=13).

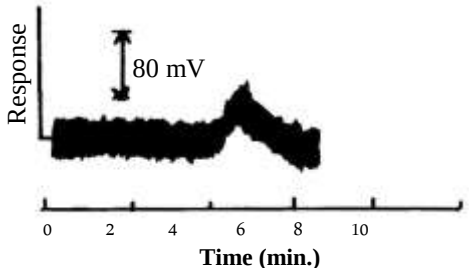

Figure (7): Chemiluminescence signal vs. time for 50 nmol.L^{-1} of histidine above distilled water background (signal enlarged 3x)

Calculation of Number of theoretical plates (N) and the Height Equivalent to theoretical plate (H); Chromatographic approach

Calculating the width of each successive peak obtained by triangulation show that 2.1 cm is the width (w). The retention time (t_R) value is 4.8 cm (Figure no.8).

Now 2.1 cm $\Rightarrow$ 2.1 min
4.8 cm $\Rightarrow$ 4.8 min

$$N = 16\left(\frac{t_R}{w}\right)^2 = 16\left(\frac{4.8}{2.1}\right)^2 = 83.59 \approx 84\,\text{plate}$$

Since: L (packed column length) = 100 cm

$$H = \frac{L}{N} = \frac{100}{84} \Rightarrow H = 1.19\text{cm}$$

μ = average linear velocity of solution in cm/sec

$$\mu = \frac{L}{t_R} \Rightarrow \mu = 0.35\,\text{cm/sec}$$

For according H_{ex} = extra column band broadening by:

$$H_{ex} = \frac{\pi r^2 \mu}{24\, D_m}$$

D_m= diffusion coefficient in the mobile phase.

Assuming D_m = 1 approximation approach

r = radius of the tube

$$\text{so } H_{ex} = \frac{3.14 \times \left(\frac{0.5}{10}\right)^2 \times 0.35\,\text{cm/sec}}{24 \times 1\,\text{cm}^2/\text{sec}} \Rightarrow H_{ex} = 0.00011\,\text{cm}$$

The height equivalent to theoretical plate

$= H_{column} + H_{ex}$

$= (1.19 + 0.00011)$ cm

All values of t_R for different concentrations are equivalent to any other concentration and the width of the response obtained by triangulation is constant (2.1 cm = 2.1 minutes) all measurements were made using the flowgram shown in Fig.(8).

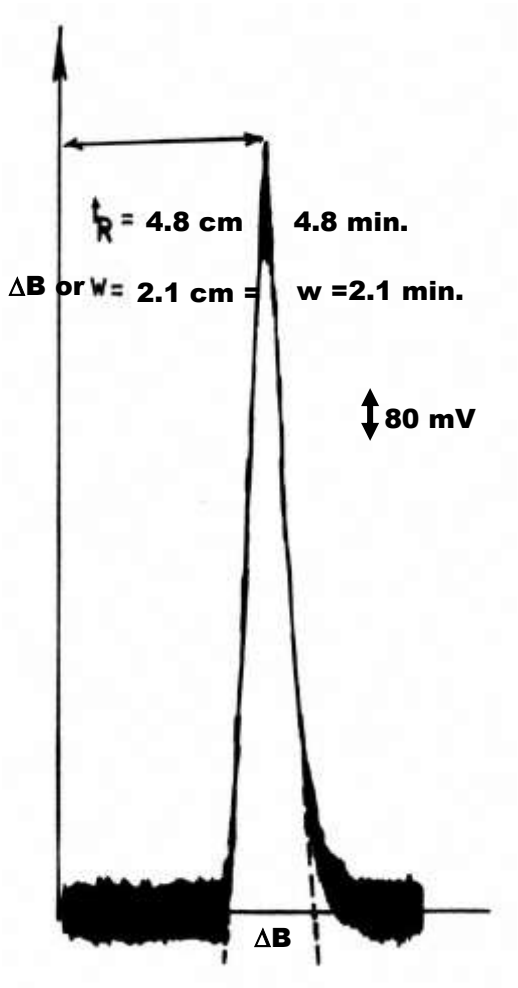

Figure (8): Chemiluminescence intensity (pA) vs. time (min) profile (3x) of normal response obtained for DL-Histidine using Histidine (80μmol.L^{-1})↔Co(II) (1000μg.ml^{-1})-Luminol (0.5 m.mol.L^{-1}) -H_2O_2 (0.5 mol.L^{-1}) system.

Discussion

The present paper introduces a unique approach for the determination of histidine via its binding to cation exchange resin through the expected mechanism of conjunction at the positive site of the molecule. An improved linearity and detection limit compared with a available literature cited in the introduction. The present paper also presented a new approach of using quadratic equation and proved to be valid at the range of concentration used. In addition it presents a new approach for simulation of chromatographic theory of calculating number of theoretical plate and the height equivalent to theoretical plates. Simplicity and sensitivity of the method will lead to further study for any useful application. Further work is in progress.

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طريقة جديدة كيمتألقية- بالحقن الجرياني المستمر لتقدير الهستادين عبر راتنج مبادل ايوني محمل بايون الكوبلت(II)

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(تاريخ الاستلام: ٣ / ٦ / ٢٠٠٩ ، تاريخ القبول: ١٨ / ١ / ٢٠١٠)

الملخص

يقدم هذا البحث مقاربة جديدة كلياً لتقدير عقار DL-Histidine بطريقة حساسة وبسيطة تعتمد الطريقة على استخدام راتنج مبادل كاتيوني محمل بايون الكوبلت(II). يتم استبدال جزيئة DL-Histidine بالكوبلت المحمل على سطح تراكيب الراتنج مطلقاً ايون الكوبلت(II).

ايون الكوبلت(II) يحفز تكسير جزيئة البيروكسيد مؤديا الى تفاعل البريق الكيميائي. طاقة البريق الكيميائي تقاس واعزيت الى التركيز باستجابة منفردة واضحة المعالم وكانت الخطية للمدى (٠-١٠٠٠) مايكرومولار لها معامل ارتباط ٠,٩٩٣٥ مع معامل التقدير ٩٨,٧١% وحد كشف ٥٠ نانومولار باستخدام حجم عينة ٢٥ مايكرولتر. بينما كان معامل الارتباط للمدى (٠-١٠٠٠) مايكرو مولار مساوياً ٠,٩٩٨١ اما معامل التقدير فكان ٩٩,٦٣% لمعادلة من الدرجة الثانية.

معالجات مفصلة للبيانات تم اجراءها واجراء تحليل المتباينات (ANOVA) لكلا المعادلتين وتم الاستنتاج بامكانية استخدام كلا المعادلتين للمدى المعطى من التراكيز.

تم في هذا البحث ايضاً مقاربة حسابية للكروماتوغرافيا لعدد الصفائح النظرية المفترضة والارتفاع المكافئ للصفيحة النظرية ايضاً وتم حساب H_{ex} على افتراض ان معامل التنافذ الجزيئي مساوياً الى الواحد كتقريب.

الطريقة لازالت في مرحلة الدراسة والدراسات الموسعة قيد البحث.