------Jou. Raf. Sci., Vol. 21, No.4 pp 11-18, 2010------

Measurement of Glutamate-Oxalacetate Transaminase Activity in Human Blood Serum Using Differential Pulse Anodic Stripping Voltammetry

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(Received 23 / 6 / 2008 ; Accepted 13 / 7 /2010)

ABSTRACT

A new electrochemical method for measuring the activity of glutamate-oxalacetate transaminase (GOT) in human blood serum was developed based on the appearance of a voltammetric peak current of oxalacetate at -0.4 V vs. (Ag/AgCl, 3 mol/L KCl) as a reference electrode using phosphate buffer (pH 7.4) as a supporting electrolyte. It is successfully applied for measuring GOT activity in certain diseases such as: Liver cirrhosis and Myocardial infarction, as well as normal cases. The results of our proposed method were compared with those obtained from colorimetric method for 38 specimens of human blood serum. A good relationship between the two methods were obtained with a correlation coefficient (r = 0.9976).

قياس فعالية انزيم كلوتاميت-اوكزالواسيتيت ترانس امينيز في مصل دم الإنسان باستخدام فولتامتري الانتزاع الانودي النبضي التفاضلي

الملخص

يتضمن البحث إيجاد طريقة كهروكيميائية جديدة لقياس فعالية أنزيم كلوتاميت-اوكزالواسيتيت ترانس امينيز في مصل الدم البشري باستخدام تقنية فولتامتري الانتزاع الانودي التفاضلي. حيث تعتمد هذه الطريقة على ملاحظة الزيادة الحاصلة في تيار موجة الاختزال العائدة لمادة الاوكزالواسيتيت المتكونة خلال التفاعل المحفز بواسطة هذا الإنزيم. حيث ظهرت هذه الموجة عند جهد (0.4-) فولت ضد قطب المرجع (فضة / كلوريد الفضة، 3 مولاري كلوريد البوتاسيوم) باستخدام محلول الفوسفات المنظم ذي الرقم الهيدروجيني 7.4 وقد تم تطبيق هذه الطريقة لقياس فعالية هذا الانزيم باستخدام 38 عينة من عينات مصل دم لأشخاص أصحاء واخرين مصابين بحالات مرضية معينة مثل: تليف الكبد المزمن و الاحتشاء القلبي.

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*تم إلقاءه بالمؤتمر العلمي الأول للكيمياء المنعقد بتاريخ 22-23 نيسان 2008 في قسم الكيمياء / كلية العلوم/ جامعة الموصل

وقد تم الحصول على تطابق خطي بين النتائج المستحصلة بطريقتنا المقترحة مع النتائج المقاسة بالطريقة اللونية وكانت العلاقة خطية بينهما وبمعامل ارتباط مقداره (r = 0.9976).

INTRODUCTION

Glutamate-oxalacetate transaminase GOT also known as aspartate aminotransferase (AST) has the ability to catalyze the following enzymatic reaction as shown in equation (1) :

$$\alpha - \text{Oxogltarate} + \text{L} - \text{Aspartate} \xrightarrow{\text{GOT}} \text{L} - \text{Glutaminate} + \text{Oxaloacetate} \ldots\ldots\ldots\ldots (1)$$

Transamination reactions play significant role in intermediary metabolism. They require pyridoxal phosphate as a coenzyme for their catalytic activity (Nelson and Cox, 2005). Aspartate aminotransferase found in many animals and plants tissues and its activity is especially high in mammalian heart and liver (Zilva and Pannal, 1984). The enzyme exists in two distinct isoenzyme forms in mammalian tissues, the mitochondrial form and the cytoplasmic form. However, GOT levels in human blood serum are used in clinical diagnosis of liver and heart diseases (Burtis and Ashwood, 1994). Serum GOT levels in healthy cases are low, but the levels are significantly elevated in a number of clinical conditions such as acute and chronic hepatitis, obstructive jaundice, carcinoma of liver, myocardial infarction and muscular dystrophy (Sherwin and Sohenes, 1996). Therefore, determination of serum GOT level has great clinical and diagnostic significant. It has applications in couple enzyme reactions for the measurement of metabolite levels in biological fluids (Kim *et al.,* 2001; Maitra *et al.*, 2005).

Several methods were available for measuring GOT activity, the colorimetric method based on monitoring the concentration of oxalacetate hydrazone formed with 2,4-dinitrophenylhydrazine (Reitman and Frankel, 1957). Stripping voltammetry is a very sensitive electroanalytical technique that used for trace analysis. It has a lowest determination limits in comparison with other commonly used electroanalytical techniques. The three most commonly used variations of stripping voltammetry used for quantitative determination of electroactive compounds are: anodic stripping voltammetry (ASV), cathodic stripping voltammetry (CSV), and adsorptive voltammetry (AdSV) (Wang, 1990). Due to the high sensitivity and selectivity of stripping voltammetry, it has the ability to use it with another voltammetric technique such as square-voltammetry (SWV) or differential pulse voltammetry (DPV). (Brett and Bret, 1993)

In the present work, differential pulse anodic stripping voltammetry (DPASV) was used for measuring GOT activity in the real specimens of human blood serum as a best voltammetric technique for this purpose.

EXPERIMENTAL

Apparatus

The electrochemical studies were carried on EG&G 384 B Polarographic Analyzer with hanging mercury dropping electrode (HMDE) as a working electrode, (Ag/AgCl, 3M KCl) as a reference electrode, and Pt wire as an auxiliary electrode. The colorimetric studies were performed on Cecil UV-Vis CE 10211 digital single beam spectrophotometer.

Temperature control was performed using thermostatic water bath type radiometer VTS 13 for electrochemical measurement, and thermostatic water bath model SB 10 from British Grants Instruments Limited for colorimetric measurements.

Chemicals and reagents

The GOT contains phosphate buffer (100 mmol/L, pH 7.4), L-aspartate (100 mmol/L), α-oxoglutarate (2.0 mmol/L) in a total volume of 100 ml Sodium Hydroxide (4.0 N) in a total volume of 100 ml, and 2,4-dinitrophenylhydrazine (2.0 mmol/L) in a total volume of 100 ml were supplied from Randox laboratories Ltd. Company (Randox, Cat. No. AS 147). (0.2 mol/L) Phosphate buffer pH 7.4 was prepared by dissolving 3.4836 g of dipotassium hydrogen phosphate K_2HPO_4 and 2.7218 g of potassium dihydrogen phosphate KH_2PO_4 in a total volume of 100 ml deionized distilled water. (10^{-3} mol/L) oxalacetate stock solution was prepared freshly by dissolving 0.0013 g of oxalacetic acid in a total volume of 100 ml deionized distilled water. (0.4 N) NaOH solution was prepared freshly from NaOH stock solution. For quality control tests, three human assayed serum levels were prepared freshly by dissolving each vial of lypophilized serum with exactly 5 ml of deionized distilled water, then waiting for 30 min to complete dissolving process by keeping all of them out of light before use. All real specimens of human blood serum were collected from The Republic Hospital in Mosul, and then they are prepared and assayed within an hour, otherwise serum samples should be kept frozen.

Procedure

For voltammetric measurements, three-electrode cell has contained 5 ml of 0.2 mol/L phosphate buffer pH 7.4 and 0.1 ml of stock solution GOT buffer. The enzymatic reaction was initiated by addition 0.1 ml of human blood serum, the solution was de-aerated for 30 s using N_2 gas and the voltammogram was recorded after affixing the potential range from -0.1 to-0.6 V vs. (Ag/AgCl). The activity of GOT was determined depending on the increment in the current value of the reduction wave of oxalacetate that produced through the enzymatic reaction which appeared at -0.4 V vs. (Ag/AgCl). Moreover, the electrochemical cell was thermostated at 37^{o}C.

For colorimetric method, the sample cuvette contained 0.5 ml of GOT buffer and 0.1 ml of a real sample of human serum thermostated at 37^{o}C for 30 min, then 0.5 ml of 2,4-dinitrophyenylhydrazine was added and left for 20 min at 20^{o}C for complete reaction, and 5.0 ml of 0.4N NaOH was added finally to complete the colorimetric reaction. The absorbance of sample (A_{sample}) was measured at 546 nm against a blank solution.

RESULTS AND DISCUSSION

Voltammetric behavior of oxalacetate produced during GOT enzymatic reaction

The optimum conditions were examined using DPASV as shown in Tables (1-4) as follows: deposition time 0 s, conditioning time 10 s, equilibrium time 5 s, and scan rate 400 mV/s. The voltammetric cell was thermostated at 37^{o}C, the solution de-aerated by passing N_2 gas through it for 240 s. The calibration curve was constructed using serial additions of 10^{-3} mol/l oxalacetic acid solution, the linearity appeared in the range from 0 to 0.2 ml. The linear equation is: $y = 122.2x - 0.0005$; where y is the reduction peak current of oxalacetate, Ip (nA) and x is the volume of oxalacetic solution (ml), as shown in Fig. (1). The GOT activity in human blood serum was calculated using equation (2) in order to detect the

activity of GOT in the real specimens of human blood serum using DPASV. However, one unit of GOT activity (U/l) is equal to the rate of production of one micromole of oxalacetate per one minute under the assay conditions.

$$\text{GOT Activity (U/l)} = \left[\frac{x \times 0.001}{5.2+x}\right] \times 10^6 \quad \ldots\ldots\ldots\ldots (2)$$

According to the enzymatic reaction of GOT, the oxalacetate which has been produced during this reaction by using our proposed method DPASV gives us the differential pulse anodic stripping voltammogram at -0.4 V vs. (Ag/AgCl) in phosphate buffer pH 7.4 after human blood serum addition as shown in Fig. (2B). Moreover, Fig. 2A represents the differential pulse anodic stripping voltammogram for phosphate buffer pH 7.4 before human serum addition; this indicates the major role of oxalacetate produced through the enzymatic reaction.

Table 1: Effect of deposition time on the reduction current of oxalacetate

Deposition time (s)	0	2	4	6	8	10
Ip (nA)	10.880	10.431	9.910	9.880	9.730	9.720

Table 2: Effect of conditioning time on the reduction current of oxalacetate

Conditioning time (s)	0	2	4	6	8	10
Ip (nA)	10.900	11.480	11.490	11.700	11.730	12.340

Table 3: Effect of equilibrium time on the reduction current of oxalacetate

Equilibrium time (s)	5	7	9	11	13	15
Ip (nA)	11.550	11.510	11.410	11.080	10.570	10.030

Table 4: Effect of scan rate on the reduction current of oxalacetate

Scan rate (mV/s)	100	200	300	400
Ip (nA)	9.390	10.720	11.080	11.200

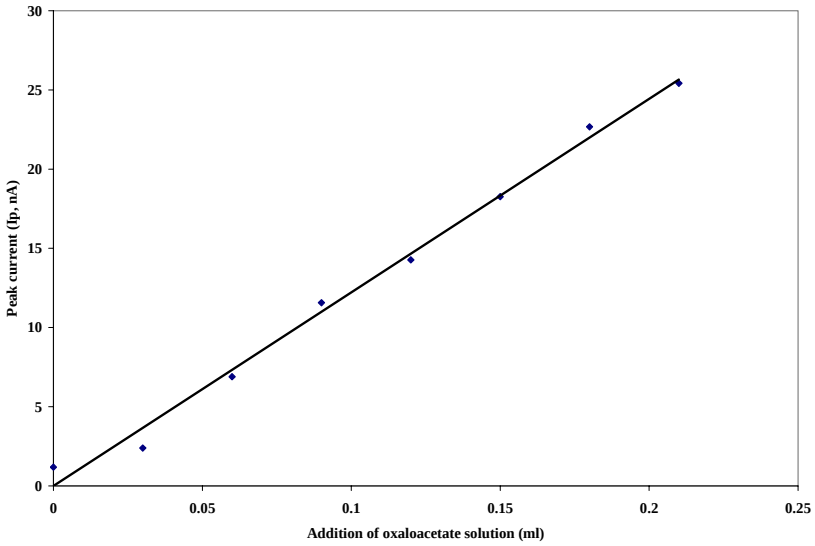

Fig.1: Calibration curve between the reduction wave current value of oxalacetate(Ip) and the series volume additions of 10^{-3} mol/L oxalacetate solution**.**

Effect of human blood serum amount

0.1 ml of a real specimen of human blood serum was selected as the optimum value in our proposed method DPASV. A set of additions ranging from 0.02 to 0.1 ml of human serum were added into the voltammetric cell. The later contains 5 ml of phosphate buffer pH 7.4 and 0.1 ml GOT buffer, respectively. The optimum value gives a maximum value of the reduction wave of oxalacetate at -0.4 V vs. (Ag/AgCl) as shown in Table 5.

Table (5): Effect of human blood serum amount on the reduction wave of oxalacetate**.**

Volume addition of serum (ml)	0.02	0.04	0.06	0.08	0.10
Ip (nA)	6.180	7.010	9.190	11.150	11.330

Comparison study between DPASV and colorimetric methods

The comparison between our proposed method DPASV and colorimetric method were carried out using 38 samples which were divided into normal cases and patients suffering from different diseases such as: Liver cirrhosis and Myocardial infarction. Table 6 shows the data which showed a consistent difference in the GOT activity between our proposed method and colorimetric method in normal and patient cases. Figure 3 displays the linear relationship for measuring GOT activity in human blood serum using DPASV and colorimetric methods with correlation coefficient (r = 0.9976). The latter indicates a good agreement between these methods. The relationship between two methods could be represented by equation (3):

GOT activity by DPASV method = [-0.2585+(0.7263*GOT activity by colorimetric method] ……..…. (3)

Table 6: GOT activity measured by DPASV and colorimetric methods in normal and patient cases.

Samples	GOT activity by DPASV method (U/l)	GOT activity by colorimetric method (U/l)
Normal (n = 21)	5.123	6.475
Liver cirrhosis (n = 8)	25.235	37.788
Myocardial infarction (n=9)	50.495	69.068

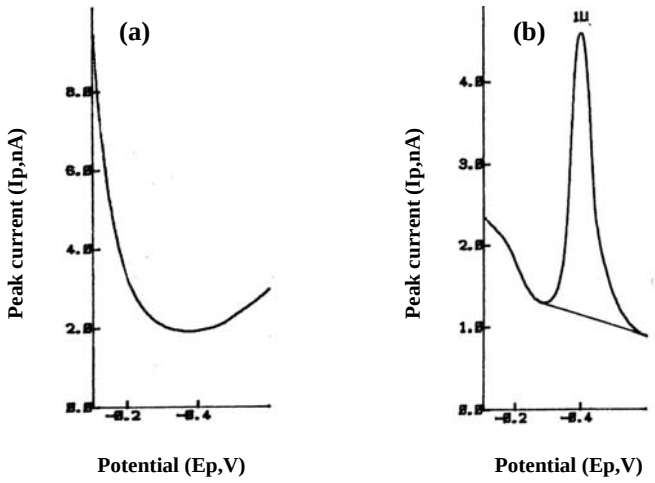

Fig. 2: Differential pulse anodic stripping voltammograms of oxaloacetate in phosphate buffer pH 7.4 as: (a) before human blood serum addition, (b) after human blood serum addition.

Quality control study

To examine the accuracy and reproducibility control from the DPASV and colorimetric methods, quality controls Levels 1, 2 and 3 were assayed respectively. Table 7 shows that the results are displayed a good agreement between our proposed method DPASV and colorimetric method.

Table 7: Quality control of GOT activity in human serum measured by DPASV and colorimetric methods.

Quality control vials	Range (U/l)	GOT activity measured by DPASV method (U/l)	GOT activity measured by colorimetric method (U/l)
Level 1 human serum	11-17	16	11.4
Level 2 human serum	19-29	25	17.9
Level 3 human serum	45-70	60	43.3

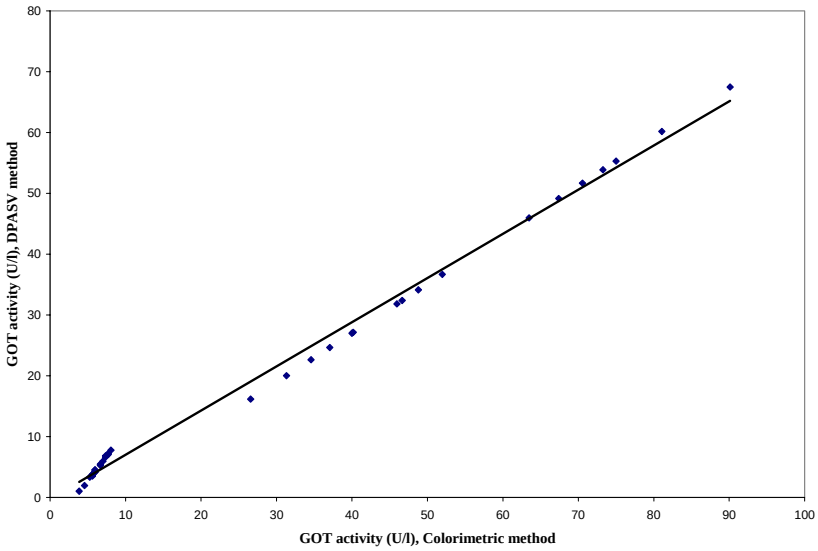

Fig. 3: The linear relationship between DPASV and colorimetric methods that used for determination of serum GOT activity in normal and patient cases.

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