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# A study to evaluate the relationship between methods of measuring uric acid levels electrochemically and the traditional method

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**Abstract**

This paper describes a method for determining uric acid in urine electrochemically through its reaction with uricase (UOx). This procedure is based on the changes in electrical potential associated with the enzymatic reaction of the working solution with uric acid as the reaction occurs, depending on the amount of decrease in the wavelength of the working solution with the addition of uric acid.

When electrochemical measurements of the study sample were performed in phosphate buffer solution (pH 7.0), the appearance of three reduction peaks was observed. The first wave, which is the main reduction wave, was adopted at a voltage of (-0.32) volts. These values were measured on the reference electrode (calomel reference) after applying a voltage ranging between (-0.8-0) volts. As for the rest of the waves, they were secondary and insensitive, so they were not adopted.

The results of electrochemical methods were compared with those obtained by conventional methods of measuring uric acid using spectrophotometry. No systematic errors were observed, but the results were largely identical, taking into account that electrochemical methods gave better results compared with the spectroscopic method is economical, accurate and fast

**Keywords:** Uric acid, Electrochemical Method, Kidney Disease, Spectroscopy

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## عنوان دراسة لتقييم العلاقة بين طرق قياس مستويات حمض اليوريك الكهروكيميائي والطريقة التقليدية

**ا.م.د.ايمان عبدالمنعم الجوادي [1] ، ا.م.د.هيثم عبدالوهاب الوهب [2]**

**المستخلص**

يصف هذا البحث طريقة تحديد حمض اليوريك في الادرار بطريقة كهروكيميائية من خلال تفاعله مع اليوريكيز (UOx). يعتمد هذا الإجراء على التغيرات في الجهد الكهربائي المرتبطة بالتفاعل الأنزيمي لمحلول العامل مع حمض اليوريك عند حدوث التفاعل، وذلك اعتمادًا على مقدار الانخفاض في الطول الموجي لمحلول العامل مع إضافة حمض اليوريك.

عندما تم إجراء القياسات الكهروكيميائية لعينة الدراسة في محلول منظم الفوسفات (الرقم الهيدروجيني 7.0)، لوحظ ظهور ثلاث قمم اختزال. تم اعتماد الموجة الأولى وهي موجة التخفيض الرئيسية عند جهد (-0.32) فولت. تم قياس هذه القيم على القطب المرجعي (مرجع الكالوميل) بعد تطبيق جهد يتراوح بين (-0.8-0) فولت. أما بقية الموجات فكانت ثانوية وغير حساسة فلم يتم اعتمادها.

وتمت مقارنة نتائج الطرق الكهروكيميائية مع تلك التي تم الحصول عليها بالطرق التقليدية لقياس حمض اليوريك باستخدام القياس الطيفي. لم يلاحظ أي أخطاء منهجية ولكن النتائج كانت متطابقة إلى حد كبير، مع الأخذ في الاعتبار أن الطرق الكهروكيميائية أعطت نتائج أفضل مقارنة بالطريقة الطيفية فهي اقتصادية ودقيقة وسريعة.

**الكلمات المفتاحية :** حمض اليوريك، الطريقة الكهروكيميائية، أمراض الكلى، التحليل الطيفي

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## Introduction

The principle of measuring uric acid in a urine sample is based on electrochemical methods based on a drop of mercury dripping [1], based on the enzymatic method [2]of following up on measuring the decrease in wavelength. (Working

solution) prepared according to the enzymatic method by adding uric acid [3], which gives a clear reduction wave [4] as shown in the following equations [5]:

$$\text{Uric acid} + O_2 + 2H_2O \xrightarrow{\text{Uricase}} \text{allantoine} + CO_2 + H_2O_2 \quad (1)$$

$$2H_2O_2 + \text{DHBS} + \text{4-aminoantipyrine} \xrightarrow{\text{Peroxydase}} \text{red quinine} + H_2O + HCl \quad (2)$$

The measurement by this enzymatic method [6] was made by adding (0.5) milliliters of the ready-made buffer solution of uric acid to the buffer solution of phosphate (pH 7.0). It was observed that three reduction peaks appeared [7]. The first is the main reduction wave at a voltage of (-0.32) volts. The other two reduction peaks were not taken into account in subsequent measurements [8]. Due to their lack of sensitivity to interaction, these values were measured against the reference electrode (Calomel reference) after applying a voltage ranging between (-0.8-0) volts [9], as shown in the figure (1).

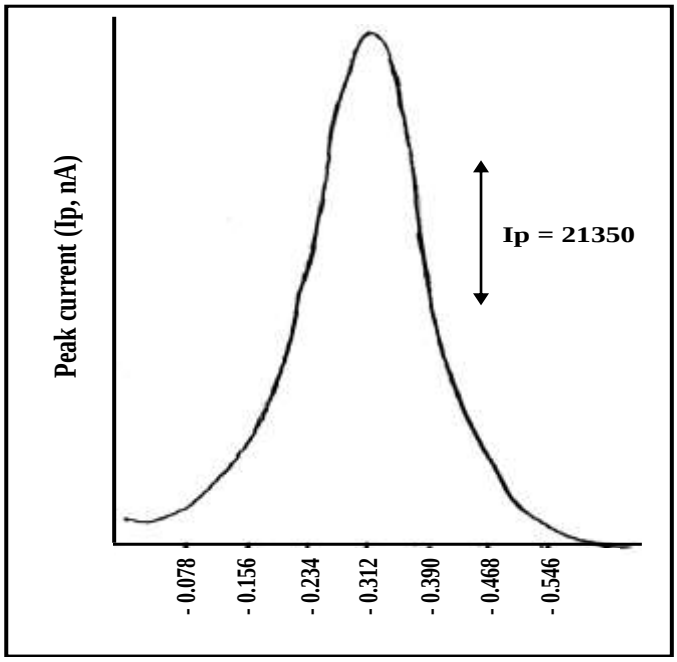


**Figure (1): shows the reduction wave of the working solution of uric acid in the urine sample - the enzymatic method**

**Method**

Method of measurement using DPP technique with sample:

1. (9.5) ml of disodium phosphate buffer (pH = 7.0) was taken in the measuring cell (clean and dry). The measurement can be performed for reference (Blank).

2. (0.5) ml of the components of the uric acid buffer solution were added to the measuring cell and the solution was shaken to homogenize it.

3. Add (0.05) ml of the urine sample. The measurement is performed after shaking to give the solution a chance to react, and the measurement is then performed. Then the

measurement is repeated again 15 minutes after adding the sample.

## Results and Discussion

### Optimization of the Conditions

The optimum conditions for the study of polarogram were determined using the enzymatic reaction, which gave the highest value of the diffusion current (Ip) and the best shape of the reduction waveform of the products returned for the determination of uric acid, which included studying the effect of pH, the fall time of the mercury drop, and the pulse amplitude, by adding (9.5) milliliters of Phosphate buffer solution with a pH of (pH 7.0), adding (0.5) milliliters of the ready-made working solution.(working solution) and uric acid at a concentration of (4.97 x 10-6) molarity, after which the diffusion current (Ip) is read at a voltage of (-0.305) volts, as these conditions were determined under the influence of the following factors:

### Effect of pH

To determine the best pH number on the reduction voltage (Ep) and the propagation current (Ip) of the enzymatic reaction reducers by uric acid, the differential pulse polarography (DPP) of the reduction products was recorded at (-0.305 volts) Where the measurement range is between (6-9) for the solution of the phosphate buffer, as in Table (1) which shows the results obtained.

**Table (1): Shows the digital effect on the specific products of uric acid - the enzymatic method using the (DPP) technique.**

| pH | Ip (nA) | Ep (V) |
|---|---|---|
| 6 | 19400 | -0.293 |
| 7 | 21300 | -0.305 |
| 8 | 14500 | -0.339 |
| 9 | 14250 | -0.349 |

It is noted from Table (1) that the reduction potential increases by a negative value with increasing pH. When drawing the relationship between pH and voltage (Ep), a straight line with a slope of (-0.0202) was obtained, as in the figure (2).

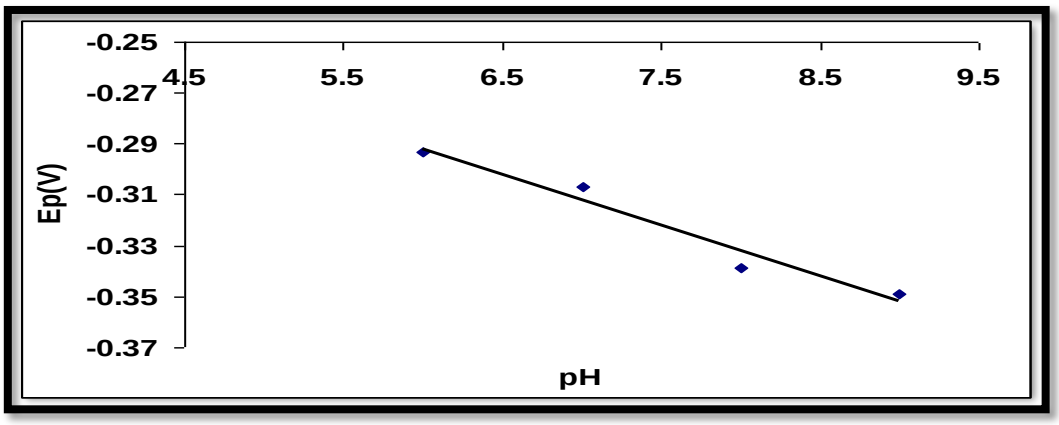


**Figure (2): The relationship between (Ep) and pH (enzymatic method using DPP**

**Effect of Drop time**

The effect of drop drop time with voltage was studied by conducting experiments to determine the best time for drop drop, and the results obtained were shown in Table (2). The best value for the droplet fall time was (2.0) seconds, as it gave the best shape and the highest value for the diffusion current. This value was fixed and used in clinical applications to measure uric acid. as show in Table (2)

**Table (2): Shows the effect of drop drop time on the diffusion current (Ip) of the products returning uric acid - the enzymatic method using the differential pulse polarography (DPP) technique.**

| Drop time (sec.) | Ip (nA) | Ep (V) |
|---|---|---|
| 0.4 | 270 | - 0.280 |
| 0.6 | 330 | - 0.300 |
| 0.8 | 380 | - 0.305 |
| 1.0 | 435 | - 0.308 |
| 1.2 | 485 | - 0.310 |
| 1.4 | 540 | - 0.310 |
| 2.0 | 710 | - 0.310 |
| 3.0 | 630 | - 0.323 |

**Effect of Pulse Amplitude**

The effect of the pulse amplitude on the diffusion current (Ip) was studied by recording differential pulse polarograms of uric acid using the pulse amplitude ranging from (20-100) mV, and the results of the diffusion current (Ip) were as shown in Table (3).The best value for the pulse amplitude is (100) millivolts.This value has been proven and used in clinical applications to measure uric acid.

**Table (3): Shows the effect of the pulse amplitude on the diffusion current (Ip) and the reduction potential (Ep) of the products resulting from uric acid in phosphate buffer solution (Phosphate buffer) at pH (pH = 7) - enzymatic method**

| Pulse amplitude | Ip (nA) | Ep (V) |
|---|---|---|
| 20 | 85 | - 0.265 |
| 40 | 180 | - 0.299 |
| 60 | 305 | - 0.306 |
| 80 | 540 | - 0.309 |
| 100 | 840 | - 0.310 |

**Effect of working solution concentration**

After determining the optimal conditions through previous experiments, the effect of the working solution consisting of buffer phosphate solution (pH 7.0) with a concentration of (150) mmol/L, peroxidase enzyme with a concentration of (660 U/L) and 4-amino antipyrene was studied.(4-aminoantipyrine) with a concentration of (1)

mmol/L, the uricase enzyme with a concentration of (90 U/L), and dichloro-2-hydroxybenzene-sulfonic acid (DHBS) with a concentration of (20) mmol/L, These components were mixed well, which as a whole represents the working solution used to measure uric acid. Table (4) shows the results of the current (Ip) and voltage (Ep) with increasing amounts for the working solution.

**Table (4): shows the effect of adding the working solution to measure uric acid - enzymatic method. Using differential pulsed polarography (DPP) technology**

| Phosphate buffer (ml) | Working solution (ml) | Ip (nA) |
|---|---|---|
| 9.9 | 0.1 | 16400 |
| 9.8 | 0.2 | 18800 |
| 9.7 | 0.3 | 19500 |
| 9.6 | 0.4 | 20550 |
| 9.5 | 0.5 | 21350 |
| 9.4 | 0.6 | 20850 |
| 9.3 | 0.7 | 20150 |

The addition of (0.5) milliliters of working solution was chosen with (9.5) milliliters of buffer phosphate solution. This additive was used in clinical applications to measure uric acid in urine.

**Effect of Quantity of Urine on uric acid measurement**

The effect of diuretic concentration on the diffusion current (Ip) of uric acid was studied by recording the differential pulse polarogram of the working solution. Gradual amounts of diuretic were added, ranging from (10-60) microliters. Table (5) shows the values obtained. the best value for measuring urine is (50) microliter, as it gave the best results and stable values for effort, as this addition was adopted in clinical applications.

**Table (5): shows the effect of adding the amount of urine on measuring uric acid - the enzymatic method using the (DPP) technique**

| Addition of urine (□l) | Ip (nA) |
|---|---|
| 10 | 200.0 |
| 20 | 650.0 |
| 30 | 1000 |
| 40 | 1800 |
| 50 | 2950 |
| 60 | 2600 |

**Effect of Time**

The effect of time on the diffusion current (Ip) was studied by recording differential pulse polarograms of a solution containing (9.5) milliliters of phosphate buffer solution (pH = 7), adding (0.5) milliliters of working solution, and adding (0.05) milliliters of diuretic acid, where The study was conducted at different times, and Table (6) shows the values obtained. the best measurement time is (10) minutes for the best reduction waveform within the time studied, and thus this time was relied upon in subsequent experiments**.**

**Table (6): shows the effect of time and diffusion current on the differential pulse polarograph to measure uric acid - enzymatic method**

| Time (sec) | Ip (nA) |
|---|---|
| 1 | 115000 |
| 5 | 113000 |
| 10 | 108900 |
| 15 | 108900 |
| 20 | 109000 |

**The standard curve**

The differential pulse polarogram was measured for a solution containing different concentrations of uric acid, ranging from (0.999001 e-6 to 9.900990 e-6) molarity in (9.5) milliliters of phosphate buffer solution and (0.5) milliliters of working solution, after stabilizing the conditions. The optimal time for the drop to fall is (2) seconds and the pulse amplitude is (100) millivolts, as in the figure (3) .When drawing the relationship between the diffusion current and concentration, a straight line was obtained with a slope of (172185264) and a correlation coefficient equal to (0.9985294).

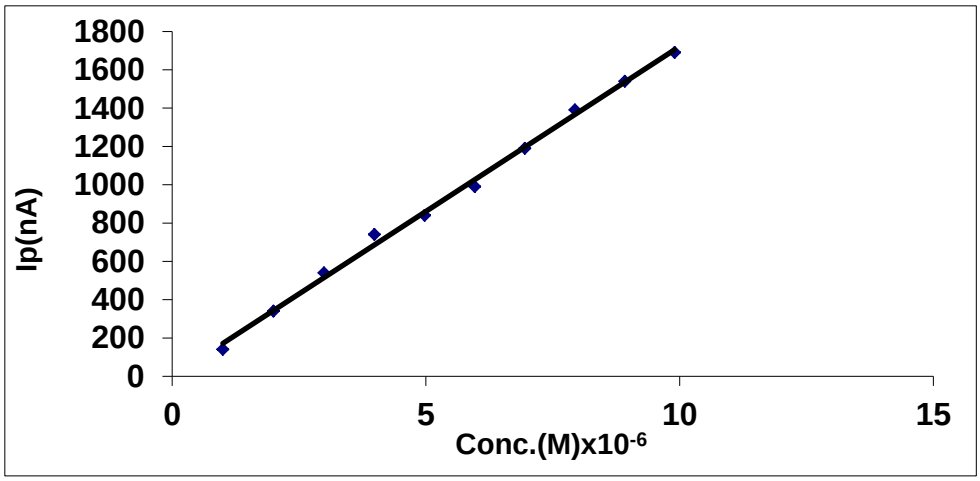


**Figure (3): shows the relationship between the concentration of uric acid with the current values resulting from the addition (by the enzymatic method) using the (DPP) technique**

**Clinical Application of Uric Acid by Enzymatic Method**

The amount of uric acid in these samples was estimated by the enzymatic method according to the proposed polarographic method (DPP) and compared with the Enzymatic method, which is one of the routine methods used to measure uric acid (mg/24hr) in urine, which produces a light pink solution in the normal state. The color is deeper in pathological conditions, as the measurement is made at a wavelength of (510) nanometers. As figure (4) shows the comparison between the proposed polarographic method and the Enzymatic method for determining uric acid in urine - the enzymatic method. For both normal conditions and in the case of kidney disease.

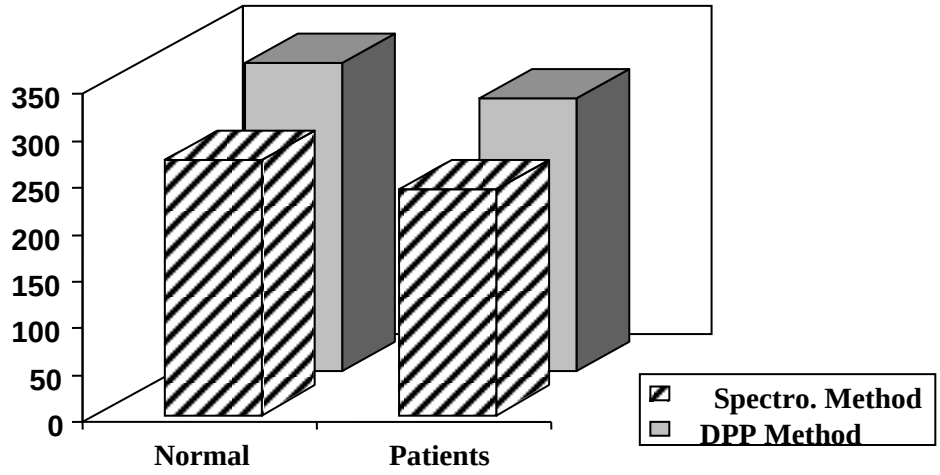


**Figure (4): shows the comparison between the proposed polarographic method (DPP) and the Enzymatic method for determining uric acid in urine samples for both normal and pathological conditions - the enzymatic method**

For the purpose of proving the accuracy of the comparison between the two proposed polarographic methods and the Enzymatic method for estimating uric acid concentration, the following correction equation (3) is used:

$$\text{DPP method} = [(52.14228) + (1.030716 \times \text{Enzymatic method})] \quad (3)$$

**Conclusions**

The use of DPP is one of the most recently proposed methods for estimating uric acid in mg / 24hr in healthy individuals with renal disease Comparison of the results obtained from this method with the Enzymatic method routinely used in pathological analyzes. It was found through the results obtained between the two methods that the proposed polarographic method gave results consistent with the results obtained in the enzymatic method with the distinction of the first method of the following: Economic, Characteristics of solutions ,Interferences, and Sensitive.

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