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URIC ACID DETERMINATION USING NANOASSAYS AND COMPARISON WITH CONVENTIONAL METHODS

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ABSTRACT

The urinary uric acid concentration (mg/24h) was indirectly determined using a sophisticated, chemically based nano-reagent method relying on differential pulsed polarography (DPP) measurement on a Dropping mercury electrode (DME), and the results were compared with spectroscopic methods. A comprehensive study of urinary uric acid concentration (mg/24h) was conducted using this nano-prepared Folin-Denis reagent, which relies on the reduction wavelength of a Folin-Denis nano-reagent prepared in the presence of uric acid. This reduction reaction with uric acid in the presence of a base resulted in two distinct reduction waves: one with a reduction potential of -0.507 V and the other with a reduction potential of -0.64 V, on a calomel electrode after applying a potential between -0.2 and -0.8 V. The integration of carbon nanotubes and nano-reagents has shown improved sensitivity, providing a robust electrochemical platform for monitoring uric acid levels associated with kidney function.

KEYWORDS: Uric acid, DPP,Carbon nanotubes, nano-reagents ,Spectroscopic method, kidney function .

INTRODUCTION

Uric acid is a biochemical compound, a byproduct of purine metabolism in the human body. It is normally produced in the liver and excreted by the kidneys in urine.It is a colorless, odorless, crystalline substance. It is soluble in water, but less so in urine. (1, 2) Normal uric acid levels in healthy individuals range from 3.5 to 7.2 mg/dL for men and 2.6 to 6 mg/dL for women(3). When uric acid levels in the body exceed the normal range mentioned above, an increase or excess of its concentration in the human blood occurs. This condition is scientifically called hyperuricemia. If the increase continues, it can develop into a disease called gout, which is a disease that damages the joints, causing severe and painful inflammation accompanied by redness and swelling of the affected area. This naturally indicates the presence of a systemic inflammatory disease (4). This often results from the primary product of uric acid, purine, and its presence in the body. Therefore, foods containing large amounts of purines will naturally increase their concentration in the bodies of those who consume them. Among the most prominent foods containing purines are red meat of all kinds. There are also studies linking high uric acid levels to individuals with a genetic predisposition, i.e., a family history, especially those who often suffer from obesity. (5,6)

In addition, high uric acid levels can lead to several negative health phenomena, including their direct effect on salt accumulation in the kidneys, causing kidney stones in some cases (7), kidney disease associated with diabetes (diabetic nephropathy) (8), cardiovascular diseases (9), and their close relationship to high blood pressure (10). In many cases and recent research, elevated uric acid levels have been closely linked to increased cholesterol levels in the body (11). Therefore, it is essential to maintain uric acid levels within a healthy range through regular monitoring of lifestyle and nutrition, particularly for individuals with conditions associated with high uric acid or a family history of such conditions(12) .Thus, it is clear that uric acid plays a significant role in influencing metabolic processes, in addition to other conditions associated with its elevation, such as gout. This can, in some cases, lead to impaired uric acid metabolism and even problems related to an individual's comfort and sleep. Therefore, it is clear from the above that it plays a significant role in influencing metabolic processes, in addition to other conditions associated with its elevation, such as gout. This may, in some cases, lead to impaired uric acid metabolism and even problems related to an individual's comfort and sleep.(13).
Hence, research has sought to measure uric acid using traditional methods(14) and chromatograph(15) and through new designs such as sensing(2,16)and electrochemical(17). Work is underway to develop these designs, using a variety of measurement methods including direct (18,19,20)and indirect (17,21,22)methods, and to improve them by adding direct additives or nano-reagents (23,24), due to their role in improving the results of electrochemical analysis, for measurements that are both rapid and highly accurate.

METHOD

Preparation of Nanoassay Solution:

To obtain a solution or suspension of carbon nanotubes, suspend 0.1–0.5 mg of nanoparticles in 50 mL of distilled water. Use ultrasound to disperse the particles to ensure a homogeneous nanoparticle size.Then, mix in a 0.5:1.5 ratio of Folin Denis reagent and use ultrasound until homogeneous for use.

Measurement using DPP with a uric acid sample:
Using an electrochemical cell, add 9 ml of pH 7.0 solution and pass nitrogen gas through for 15 minutes to measure the reference sample. Then add the prepared nanosolution with a 1:0.5 sodium carbonate ratio, pass nitrogen gas through again, and measure the nanosolution. Finally, add 0.1 ml of the previously collected urine sample for measurement.

RESULTS AND DISCUSSION

Through practical experiments to determine urinary uric acid concentration (mg/24h) using the advanced method based on chemically synthesized nano-reagents, the reduction waves were characterized in the presence of the nano-reagent and compared with the results obtained using the chemical reagent alone (15) using electrochemical methods with DPP on a Dropping mercury electrode (DME), as shown in the following figure(1)

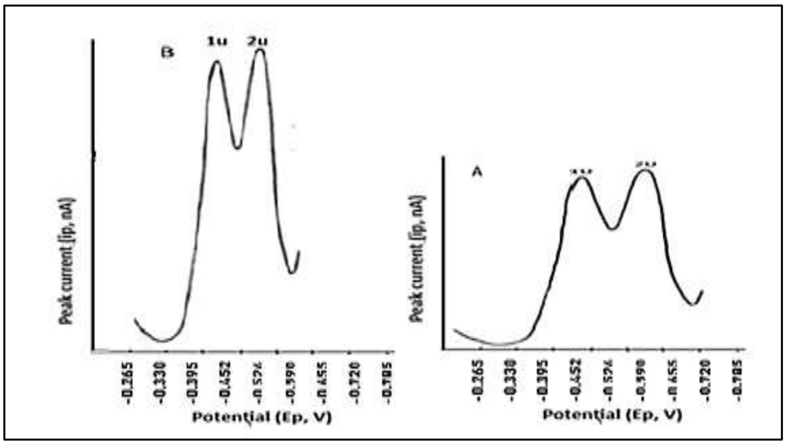

figure.1): Wave values for DPP of uric acid in A. Chemical reagent alone B. Prepared nano-reagent

Measurements or Organization of Experimental Conditions
Initially, the measurement conditions for studying the polarogram of the reduction of the prepared nano-chemical reagent in the presence of uric acid were determined. Various measurements were taken by monitoring the effect on the reduction waves of the material to achieve the best result in terms of the final waveform, focusing on increasing the resulting current at the lowest concentrations, following the procedure described above.
The buffer solution was initially identified by testing its pH. Measurements were then performed on both solutions used with uric acid: first with the chemical solution as is, and then with the prepared nanochemical reagent. The results, shown in the following figure.2, indicate that the values obtained with the nanochemical reagent were more accurate.

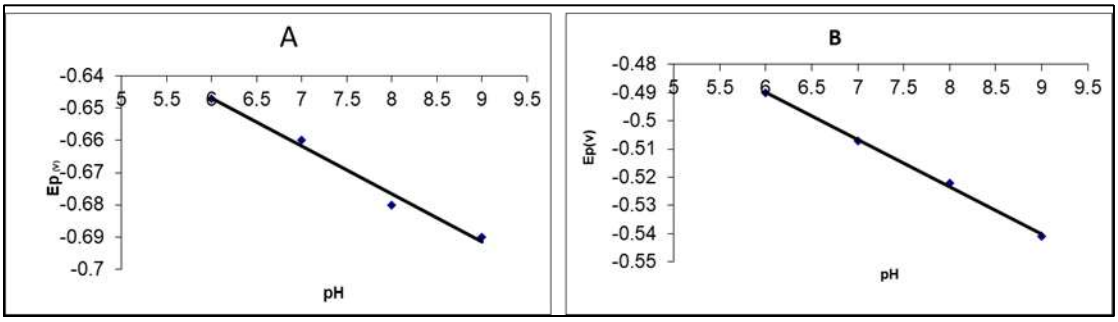

Figure.2):Polarographic relationship of pH with wave potential in:A. Chemical reagent alone B. Prepared nano-reagent

The measurements included the relationship between the time of fall of the mercury droplet (D.T.) and the voltage. Measurements were performed for both detectors (the chemical detector alone and the nano-resolution) using DPP. This effect on the reduction wave was observed with uric acid, and the optimal value of (D.T.) was determined to be 2 seconds from among the selected values ranging from 0.6 to 4.0 seconds. This value was identical for both detectors used in the experiment, with the nano-resolution detector yielding clearer wave values. To further determine the optimal conditions, the effect of changing the pulse amplitude was considered. A differential pulse polarogram was recorded to study the effect of pulse amplitude on the reduction diffusion current for values of 10–110 mV for both detectors used to determine uric acid (the chemical detector alone and the nanochemical detector). The experiment showed that the solution containing the chemical reagent alone at 100 mV yielded the best results, with a clear waveform and the highest current value. When compared to the nano-reagent, the current also increased to its highest value, producing a sharp waveform at 110 mV. This indicates the greater effectiveness of the nano-reagent in these measurements.

The reaction time and its effect on the reduction waveform of the solution were also studied in the presence of both the chemical and nano-reagents. Twelve readings were taken, ranging from 1 to 36 seconds for both experiments. The first experiment, using the chemical reagent alone, yielded optimal values at 15 seconds, the best among the results studied. The experiment using the nano-reagent, however, yielded the best current value at 12 second, further indicating the superiority of the nano-reagent and its role in improving the reaction.

Study of the Standard Curve of the Reference Solution

The reference uric acid standard curve was studied based on the parameters established in previous experiments, within the molar concentration limits (99, 9.9 x 10^{-7}, 9.90099 x 10^{-6})M, using a buffered phosphate (Ph= 0.7). The experiment was performed on both solutions, first using the chemical reagent and then again using the prepared nano-reagent. The results showed that both reagents yielded good results, exhibiting a logical increase in current with increasing concentration and a significant increase in the reading sensitivity of the nano-reagent.

This indicates a linear relationship, as shown in the figure.(3), with a correlation coefficient of 0.990 for the solution containing the chemical reagent and 0.995 for the solution containing the nano-reagent.

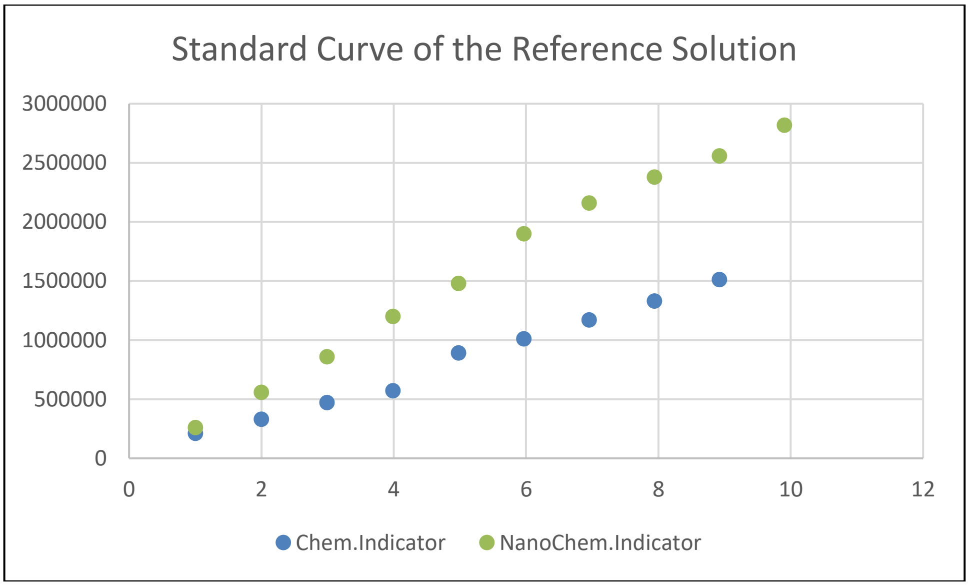

figure.3) Study of the Standard Curve of the Reference Solution

Urine Sample Standard Curve Analysis

The standard curve of urine samples collected from kidney patients (over 24 hours for each sample) was analyzed under the same conditions as the previous experiment for the standard solution and for both reagents: the chemical reagent and the nano-reagent. The results were mostly regular and showed a positive linear correlation, which was more accurate with the solution containing the nano-reagent, with a correlation coefficient of 0.99 for both, as shown in the following figure.(4)

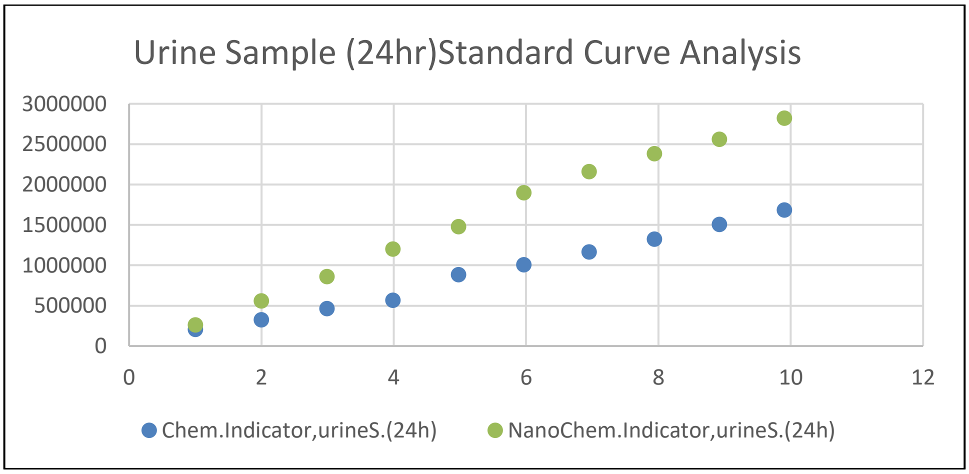

figure.4) : Urine Sample (24hr)Standard Curve Analysis

Comparison of DPP and Colorimetric Uric Acid Measurement Methods

For this study, 24-hour urine samples were collected from 15 healthy individuals and 30 patients with kidney disease using both chemical reagents (only a chemical reagent and a nano-chemical reagent) to determine uric acid concentration (mg/24h). The selected samples showed a linear relationship between the two reagents, as shown in the figure.(5)

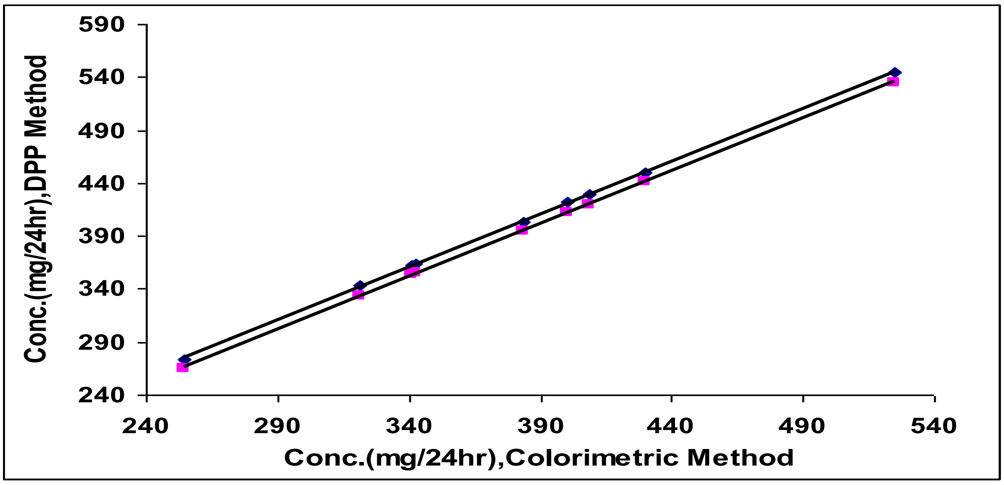

figure.5): Comparison of DPP and Colorimetric Uric Acid Measurement Methods

This study allows for a comparative relationship between the traditional method and the method used to easily determine uric acid values:

DPP = [(21.73815) + (0.995× Traditional method)] ....(1)
DPP = [(24.10361) + (0.998× Traditional method)].......(2)
DPP = [(12.32271) + (0.992 × Traditional method)].......(3)
DPP = [(13.6352 3) + (0.997 × Traditional method)].......(4)

The values for equations 1 and 2 were given for the first wave with the presence of chemical reagent 1 and nano-chemical reagent 2. Similarly, the values for equations 3 and 4 were given for the first wave with the presence of chemical reagent 3 and nano-chemical reagent 4, determining the concentration in mg/24hr of the uric acid sample. From the equations above, it is observed that the equations containing the nano-reagent yielded higher slope and gradient rates than those obtained using the reagent alone. This indicates an improved efficiency of the uric acid reaction with the nano-reagent and a significant improvement in the linearity.

CONCLUSION

The scientific advancement of increasing measurement accuracy through the development of traditional methods and the use of the polarographic search method, enhanced by nanotechnology using nanomaterials, through the development of a nanoparticle chemical reagent, will provide accurate and rapid measurements with high sensitivity compared to traditional methods. This will save considerable time and effort for those working in this field when using these improved methods.

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