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Evaluation of purified urease activity from *Proteus mirabilis* using iron oxide nanoparticles and measurement of urea concentration in blood

Abstract- *The activity of urease purified from Proteus mirabilis bacteria was estimated using gamma iron oxide nanoparticles (γ-Fe_2O_3) which were obtained as standard in size (20- 40)nm, purity 99%. The enzyme activity was estimated by incubating the pure enzyme with various concentrations of nanoparticles ranged between (1- 6) µg/ml. The results indicates a decrease in enzymatic activity with increasing of nanoparticles concentration. After that, the concentration of blood urea was measured using urease obtained from a standard kit, urease purified from Proteus mirabilis and the urease- gamma iron nanoparticles solution. The comparison was then made among the results of urea concentrations by statistical analysis using T-test. The results showed that there is no significant difference between the results obtained from urease standard kit and urease purified from the bacteria, this is due to the efficiency of urease purified from the bacteria. On the other hand, the results showed that there is a significant difference ($P \leq 0.01$) in urea concentrations obtained from urease- gamma iron oxide nanoparticles solution due to the inhibition of the enzyme which lost its activity by nanoparticles.*

Keywords: *Proteus mirabilis, urease, iron oxide nanoparticles*

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1.Introduction

Urease is a nickel containing enzyme which catalyzes the hydrolysis of urea to ammonia and carbon dioxide, it is produced by bacteria, algae, fungi and plants, the main role of urease is to allow the microorganisms to use urea as a source of nitrogen, furthermore, urease plays an important role in nitrogen cycle in plants, urease produced by bacteria, also, acts as a virulence factor in many human infections [1]. Recently, Iron oxide nanoparicles have attracted a great attention because of their unique properties and their wide applications in modern sciences, the most common iron oxide nanoparticles in biomedical applications are Magnetite (Fe_3O_4) and Maghemite (γ-Fe_2O_3) [2]. Nanoparticles have a high affinity towards some proteins since they bind to thiol groups of protein. Consequently, protein inhibition will occur. In other words the binding of thes
e particles with functional groups of enzymes will definitely lead to protein inhibition [3].

2.Aim of study

I. Inhibition of urease activity using gamma iron oxide nanoparticles (γ-Fe_2O_3).
II. Measurement of urea concentration in blood using standard kit urease, urease purified from *Proteus mirabilis* and urease– iron nanoparticles solution, then making a comparison among them.

3.Materials and methods

I.Urease.

Firstly, urease enzyme was extracted from *Proteus mirabilis* after it had been cultured in Luria broth media at 37°C for 24 hrs. Secondly, the enzyme was partially purified by ammonium sulfate precipitation according to salting out method. Then the enzyme was completely purified using ion exchange column and gel filtration column. Finally, the enzyme activity, protein concentration and specific activity of purified enzyme were estimated, and the purification fold was 11.27 and the yield was 14%, and the enzyme was saved for measuring of urea concentration in blood [4].

II. Evaluation of urease- gamma iron oxide nanoparticles activity

Firstly, 50 µl of gamma iron oxide nanoparticles suspension with various concentrations ranging from (1- 6) µg/ml were added to 250 µl of urease purified from Proteus mirabilis, the solution was left for 60 minutes at room temperature. Secondly, 250 µl of urea solution (0.167 mg/ml) was added to the solution, and incubated for 5 minutes in a water bath at 40°C, then, the reaction was stopped by adding 250 µl of 10% TCA .The solution was centrifuged at 2000 rpm for 10

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minutes. Finally, the absorbance was measured at 625 nm and the enzyme activity was quantified according to the equation below and the relationship was plotted between various concentrations of nanoparticles and enzyme activity [5,6].

$$\text{Enzyme activity (Unit/ml)} = \frac{\text{Absorbance}}{\text{slope}} \times \frac{1000}{\text{molecular weight of produt}} \quad (1)$$

Enzyme activity: is the amount of enzyme that catalyzes the formation of one micro mole of urea to ammonia per minute under defined condition of temperature and pH.

III. Measurement of urea concentration in blood

Ten blood samples were taken from healthy people of both sexes and various ages and placed in plain tubes free of anticoagulant. Serum was then separated using the centrifuge at 3000 rpm for 5 minutes. Urea concentration was measured in serum using urease from standard diagnostic kit, urease purified from *Proteus mirabilis* and the urease -gamma iron nanoparticles solution. This was done according to the colorimetric Brothelote method by calculating the amount of ammonia released from urea and interacting with the sodium nitroproside detector in the presence of sodium hydroxide, and then the absorption was measured at 625 nm.

Blood urea concentration was calculated according to the following equation:

$$C_{\text{Sample}}(\text{mg/dl}) = \frac{\text{A Sample}}{\text{A Standard}} \times C_{\text{Standard}} \quad (2)$$

Where C is the concentration and A represents the absorbance

١. *Standard diagnostic kit*

Reagent A1: consisted of sodium salicylate 62 mmol/L, sodium nitoprusside 3.4 mmol/L and phosphate buffer 20 mmol/L, pH 7.

Reagent A2: Urease

Reagent B: consisted of sodium hypochlorite 7mmol/L, sodium hydroxide 150 mmol/L

Urea standard solution 8.3 mmol/L

*These reagents were provided from BioSystems S.A. Costa Brava Company

Working solution was prepared by mixing 1 ml of reagent A2 with 24 ml of reagent A1, and then it was kept at (2-8)°C

The work solution was prepared twice again, the first one was prepared by replacing the standard kit urease with urease purified from *Proteus mirabilis*, the other one was prepared by replacing the standard kit urease with urease-iron oxide nanoparticles solution.

٢ *Measurement method*

The Brothelote method is used to measure the concentration of urea in the blood as shown in Table (1) [7,8].

3. *Statistical analysis*

The results of urea concentration in blood were statistically analyzed as (Mean± Standard error), and the differences between the results were investigated using T-test calculated with excel program.

Table 1: Measurement method of urea concentration in blood

Solutions	Blank	Standard	Sample
Work solution	1milliliter	1milliliter	1milliliter
Standard solution		10microliter	
Sample solution			10 microliter
Mix thoroughly and incubate the tubes for 10 minutes at room temperature			
Hypochlorite solution	1milliliter	1 milliliter	1 milliliter
Mix thoroughly and incubate the tubes for 10 minutes at room temperature			

4.Results and discussion

I. *Urease activity after addition of gamma iron oxide nanoparticles*

Urease activity was measured after the addition of various concentrations (1- 6) μg/ml of gamma iron oxide nanoparticles, and the solutions were left at room temperature for 60 minutes. The results shown in Figure (1) revealed that the enzyme activity gradually decreased with the increase of gamma iron oxide nanoparticles concentration. The enzyme activity was 1.3 unit/ml at the concentration of 1 μg/ml whereas the enzyme activity was 0.39 unit/ml at the concentration of 6 μg/ml, and this is attributed to the inhibition of enzyme activity because the nanoparticals have a high adsorption capacities due to their large surface areas. The binding of protein to these surfaces induces conformational change at secondary and tertiary structure, which mean the protein adsorption on the nanoparticles surface affects protein structure and function [9]. The protein adsorption is driven by various protein

forces, including Van der waals, hydrophobic and electrostatic [10].

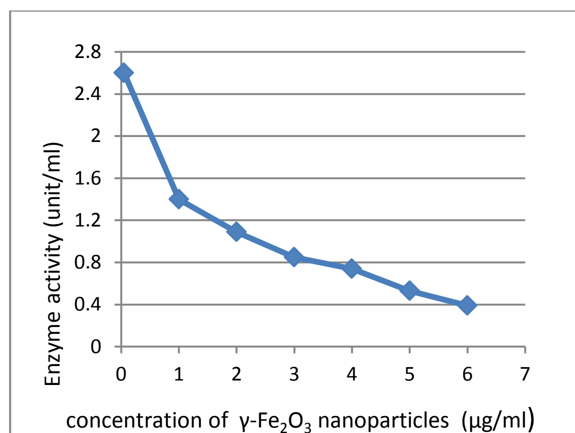

Figure 1: Effect of γ-Fe_2O_3 nanoparticles on purified urease activity from *Proteus mirabilis*

Therefore, the optimal method to describe the interaction between urease and nanoparticals is adsorption phenomenon, urease contains four surfaces with exposed histidine and several cysteine residues, the presence of these residues affect urease activity by adsorption [11], and the inhibition of urease by nanoparticles resulted from reaction of nanoparticles with sulfhydryl group of cysteine in mobile flap of the active site of enzyme, which is similar to the reaction of insoluble sulfides formation. Nanoparticles which form these sulfides are a strong inhibitors of the enzyme which requires the presence of free -SH groups which are important for active site of enzyme and enzyme activity [6].

II. Urea concentration

The concentration of urea in the blood was measured using urease standard kit, urease purified from *Proteus mirabilis*, and urease-gamma iron oxide nanoparticles solution. The results of urea concentrations explained in Table (2) showed that there is no significant difference between the concentration of urea measured using urease standard kit and urea concentration measured using purified urease. This is attributed to the efficiency of urease purified from bacteria [12], while the results showed that there is a highly significant difference ($p \leq 0.01$) between the concentration of urea measured by standard kit urease and urea concentration measured by the urease– gamma iron oxide nanoparticles solution. Similarly, there is a highly significant difference ($p \leq 0.01$) between urea concentration using urease purified from *Proteus mirabilis* and urea concentration measured by urease– gamma iron oxide as shown in Table (2). The difference is attributed to the decrease in urease activity after the addition of nanoparticles [13].

Table 2: urea concentrations in blood using standard kit urease, urease purified from *Proteus mirabilis* and urease– iron oxide nanoparticles

Urea concentration measured using standard kit urease (Mean ± Standard error)	Urea concentration measured using urease purified from *Proteus mirabilis* (Mean ± Standard error)	Urea concentration measured using urease–gamma iron oxide nanoparticles (Mean ± Standard error)
1.98 ± 24	1.6٠ ± 21.8	0.86±6.7**

P≤0.01=**

5. Conclusion

I.. The activity of purified urease from *Proteus mirabilis* bacteria was decreased after the addition of gamma iron oxide nanoparticles.

II. Purified urease was used as an alternative to the diagnostic kit to measure the concentration of urea as it proved to be as efficient as diagnostic kit.

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