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Potentiometric Urea Biosensor with High Stability by Using silver oxide Nanoparticles

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Abstract

Highly stable urea biosensor was prepared using silver oxide nanoparticles (Ag NPs), which were used in a paste with urease enzyme. The urea biosensor was prepared using a urea-selective electrode with urease-immobilized nanoparticles. The synthesized nanoparticles were characterized using scanning electron microscopy (FE-SEM) and Energy Dispersive X-ray Spectroscopy, and the formation of silver oxide was detected and confirmed using infrared spectroscopy. The potentiometric response of the biosensor was examined as a function of different urea solution concentrations,an enzymebased urea sensor through immobilization of the enzyme urease that was found to be sensitive to urea concentrations from 0.1 M to 0.000001 M. Linear sensitivity regions were observed when the electrochemical responses (electromotive Force) of the sensors were plotted vs. the logarithmic concentration range of urea from 0.01 M to 0.00001M. The proposed sensor showed a sensitivity of 59.28 mV/decade for 0.01- 0.00001 M urea and a fast response time of less than 30s was achieved with good selectivity, reproducibility and negligible response to common interferon's such as glucose, cholesterol, uric acid , lactic acid and ascorbic acid .

Keywords: Biosensors, Potentiometric, Urea, Silver oxide Nanoparticles**,** Urea enzyme.

Introduction

Urea is the final product of nitrogen metabolism in mammals. In human beings, it is a main indicator of liver and kidney activity and a marker for hemodialysis treatments. Furthermore, urea is used in many industrial processes, in agriculture and the farm industries. Thus, it is important to evaluate the level of this compound in biological fluids, in environmental matrices and food samples[1]**.** The chemical formula for urea is CO(NH2)2, and it is an organic molecule made up of two -NH2 groups and a carbonyl functional group (C=O). Determining urea levels is crucial since both high and low levels can cause liver failure and other illnesses. Therefore, there is an urgent need to detect urea in biological fluids to diagnose these diseases in their early stages. Among the various methods available for detecting urea, most are complex and require time-consuming sample pretreatment, expensive instrumentation, and trained personnel, particularly for chromatographic methods. Biosensing methods overcome these drawbacks due to their simplicity, speed, accuracy, and high sensitivity, and can also be applied to detect urea *in vivo*[2].

A straightforward, quick, sensitive, and selective method for detecting urea is offered by biosensing techniques. Because of their exceptional qualities, which include high sensitivity, high specificity, quick response, and versatility for a range of applications, enzyme electrochemical biosensors have become essential instruments in the field of biosensing. Biosensors' distinct structural features and performance metrics, including reaction time, detection limit, and detection range, are examined. By combining these crucial elements, the review emphasizes the advancements made in this area, the encouraging possibilities, and the adaptability of enzymatic electrochemical biosensors for precise urea detection[3].

The urease (Urs) enzyme is employed as a bio-receptor in urea biosensors, ensuring its sustained functioning. In recent decades, urea biosensors have employed a variety of nanoparticles, including carbon-based materials (carbon nanotubes, graphene oxide, reduced graphene oxide), conductive polymers (polyaniline, polypyrrole, etc.), and nanoparticles (zinc oxide, nickel oxide, iron oxide, titanium dioxide, tin(IV) oxide, etc.)[4]. Potentiometers are superior to their competitors some of ways, including speed, portability, affordability, high sensitivity, ease of use, and gadget simplicity. The urease enzyme has been used to create potentiometer urea biosensors. Potentiometer urea biosensors have been the subject of scientific investigations that have looked at their structural traits and performance metrics, such as detection limit, operating concentration range, response time, and lifetime, all of which are crucial for real-world applications. Since every potentiometric urea biosensor has pros and cons of its own, the sample to be examined, its range of urea concentrations, and other application-specific requirements all play a role in choosing the best approach[5]. Because nanostructures and nanomaterials have special qualities in the biosensing field, nanosensors have several major advantages because of their tiny size and high surface area to volume ratios, which enable larger signals, better catalysis, and the more Analytes pass through sensors quickly. Because of their exceptional capacity to facilitate quicker electron transfer between the electrode and the target enzyme's active site, nanostructured materials like zinc oxide, cerium oxide, tin oxide, titanium oxide, iron oxide, and zirconium oxide nanoparticles have recently been employed for the fabrication of transducer surfaces Furthermore, nanomaterials' non-toxicity, high chemical stability, and high electron transfer capacity make them promising materials for immobilizing biomolecules involved in electron or charge transfer without the need for electron mediators [6-9]. By immobilizing the selected biomolecules (enzymes) onto the nanostructured materials through various chemical interactions, the bioreceptor portion of sensitive biosensors can be readily manufactured. covalent bonding, trapping, or adsorption. More biomolecules can be carried by nanomaterials than by traditional support materials because of their large surface areas[10].In this work, we successfully demonstrated the potentiometric determination of urea using silver oxide-containing urea-selective electrodes, homogeneously fabricated in contact with silver wires, using a nanomaterial-based paste electrode method. The potentiometric response against an Ag/AgCl reference electrode was found to be linear over a logarithmic concentration range of 0.01M to 0.00001M, suitable for common serum urea levels.

Experimental details

- Materials, reagents and instruments

Urease enzyme (15,000–50,000 units/g) was purchased from Sigma-Aldrich, Urease (Urs), urea, glucose, uric acid, lactic acid, ascorbic acid, cholesterol, were purchased from Loba Chemie Pvt. Ltd. India. All aqueous solution was prepared with Mili-Q water (18.2 MΩ at 25 °C) of high purity. 100 mL of 0.1 M phosphate buffer solution (pH 7.4) was prepared to dissolve the urease enzyme for the preparation of the paste electrodes. Different concentrations of phosphate buffer solution (0.01, 0.008, 0.007, 0.005, 0.003, and 0.001 M) were prepared to study the effect of its concentration on the biosensor by applying the Henderson-Hasselbach equation. Different weights of KH_2PO_4 and KH_4PO_4 were taken, and the two substances were dissolved in an appropriate amount of distilled water, and the volume was made up to 100 mL with distilled water. Then, the phosphate buffer solution was selected with a specific molar concentration that would give the best sensitivity results, and from it, buffers were prepared to make the electrode with different pH ranges (4.5-9.5). The pH was adjusted using a specific concentration of acid and base (sodium hydroxide and hydrochloric acid). In the final step, a wide pH range was tested for the best buffer concentration, which gave good sensitivity in the previous step. All concentrations were prepared in 100 ml volumes, and the materials were prepared from (Sigma-Aldrich). Urea stock solution was prepared at a concentration of 0.1 molar, and from this concentration, diluted concentrations were prepared between (0.1, 0.03, 0.01, 0.003, 0.001, 0.0003, 0.0001, 0.00003, 0.00001, 0.000003, 0.000001 molar) by applying the dilution law, and each concentration was prepared with 100 ml of distilled water to study the best response of the prepared electrode, and the same concentrations were prepared using phosphate buffer to valuation of biosensor response and in the end stored electrode at 4°C.. All other chemicals used (Sigma, Aldrich).while instruments are HANNA Instruments pH 2110UK, Romania and reference electrode:Saturated Calomel electrode(RE-2BH),A digital pH/ion meter (EUTCH PH700) made in Singapore ,Micropipettes (10-100µl) ,Magnetic stirrer , However, Devices was FTIR Al-Nahrain Universty of Science, Energy Dispersive X-ray Spectroscopy and FE-SEM in Iran . The optimization of the electrode prior to analytical detection involves the application of the effects of several parameters, such as the effect of the supporting electrolyte, the concentration of the buffer solution, the acidity (pH) and the temperature[11].

Potential biosensors are used to detect precise concentrations of substances over a wide range of concentrations. They rely on the use of ISE to obtain the desired concentrations and analytical information. The study of urea sensors in this research is based on the response of a nanomaterial-containing paste electrode (1) in a urea solution, (2) in varying electrolyte concentrations with urea injection (study of the effect of the presence of salts on the sensitivity and linearity of the prepared electrodes. Potassium chloride was prepared at different concentrations. These concentrations were determined as follows (0.1 M, 0.01 M, 0.05 M, 0.001 M), and in the presence of urea concentrations (between 0.01 M and 0.000001) M, the working procedure was as follows: 40 ml of potassium chloride were taken in a beaker, and the result was read until confirmed: Different concentrations of urea (40 µL) were added to the selected electrodes), (3) the concentration of a buffer solution, including Tris-HCl buffer and KH2PO4 phosphate buffer, with varying pH, (4) the effect of temperature, (5) and evaluation of the biosensor response, including (1) sensitivity, dynamic range, and detection limit; (2) response time, repeatability, stability, selectivity, and application; and finally, a comparative study, which includes aspects such as (accuracy , precision and correlation.

- Preparation of Urea Biosensor

- Green synthesis of silver nanoparticles

Preparations are underway. We collected orange leaves from orange trees, cleaned them with tap water to remove suspended dirt, then washed them with distilled water and dried them in the shade. The grains were ground with an electric grinder. During two hours of heating to 80°C, 30 grams of leaf powder were added to 400 mL of deionized water. Leaf extracts were cooled at room temperature, filtered, and stored in the refrigerator at 4°C for further research. Using orange leaf extract, silver nitrate solution was prepared by dissolving 0.5 g $AgNO_3$ in 50 mL of deionized water. After adding the plant extract solution slowly to the precursor solution at 40°C, the solution was heated to 90°C. For one hour, NaOH (1M) solution was dropped into the mixture (salt solution and orange leaf extract) drop wise. A 2-hour oven-drying procedure was then conducted on the precipitate.[12]

- Preparation of Urease past electrode

Past electrodes were prepared as follows: First, 0.6 g amounts of polyaniline powder were thoroughly mixed with 0.5 g of graphite powder, and 20 microliters of paraffin oil were added and compacted to obtain a homogeneous paste, along with 0.06 g amounts of silver oxide nanoparticles, and mixed thoroughly to obtain a completely homogeneous past[13].

- Immobilization of urease in Ureas/PA/Nafion

The enzyme was then prepared by 15mg of urease dissolved in 0.5 ml of 0.1 M phosphate buffer solution at pH 7.4 and mix with 0.45mL 5%Nafion after that The 20 micro liters solution prepared was dropped[13] on the surface paste electrode .The prepared paste is filled in a 1.5 ml eppendorf and a silver wire is inserted from the top to act as an electrical conductor and complete the electrical circuit. It is left to dry for two hours. The eppendorf is pierced from the bottom and the outer surface is smoothed

-Measuring the Biosensor Response

The potentiometric response of the biosensor was evaluated as a function of urea concentration in a buffer solution to assess and select the best potentiometric potentials that yield a linear relationship and acceptable sensitivity across prepared urea concentrations (0.000001-0.1) M Figure (1). To improve the performance of the urea biosensor, the effects of the presence of salts such as electrolytes, the effects of the medium pH, buffer concentration, temperature, and incubation time on its response were studied.

The reproducibility and stability of the prepared urea biosensor were also studied. Additionally, selectivity studies were conducted using potential interfering agents present in blood, such as ascorbic acid (AA), uric acid (UR), glucose (GL), cholesterol, and glucose. Finally, the applicability of the fabricated urea sensor was tested in serum samples from different subjects whose blood urea concentrations were studied and who were exposed to ionizing radiation as workers in various Iraqi hospitals. For this purpose, the electrochemical response of urea-containing serum was compared with the laboratory method for measuring urea concentration using a Kit**.**

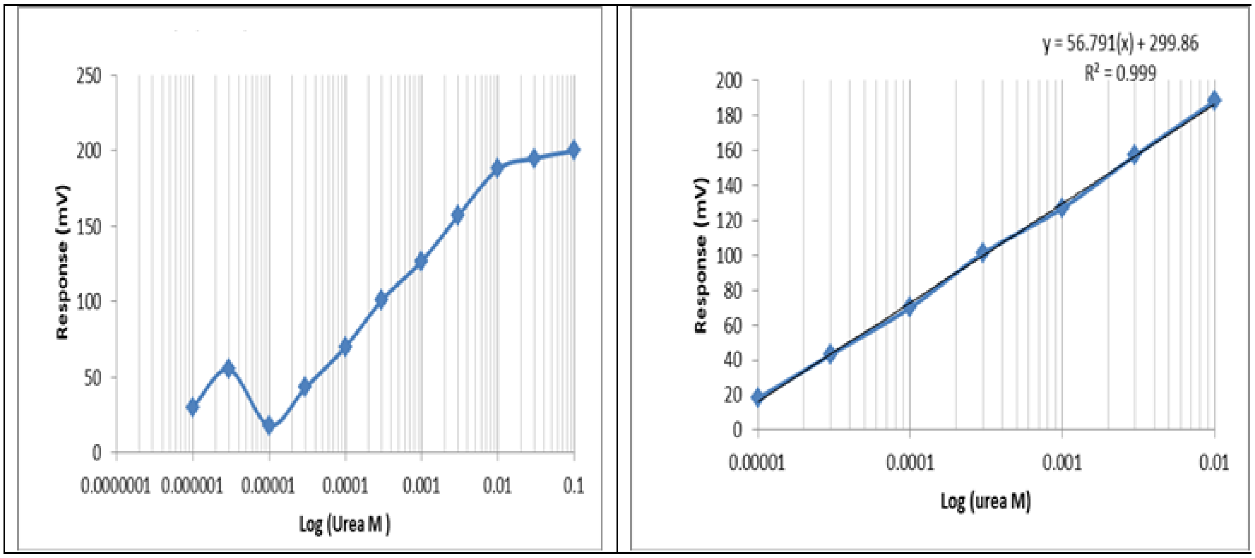

Fig. 1: Response of the paste electrode to the prepared urea concentrations.

Sensitivity of the prepared electrode was good at 56.791 mV/decade with a linearity of concentrations of (0.01-0.00001) M.

Results and Discussion

-The Characterization of Nanoparticles

-FTIR spectroscopy

The most important characteristic of the green synthesis of silver oxide nanoparticles (Ag NPs) is the metal-oxygen (Ag-O) stretching vibrations with a wavelength less than 700 cm^{-1}, in addition to the appearance of other peaks, including O–H stretching vibrations around the high-wavenumber region of 3336 cm^{-1}, resulting from the polyphenolic compounds present in the plant extract. The peak at 1637 cm^{-1} in the carbonyl region also reflects the presence of C=O stretching, indicating the involvement of aldehydes, ketones, or carboxylic acids in the reduction or stabilization process as shown in Figure (2)[14].

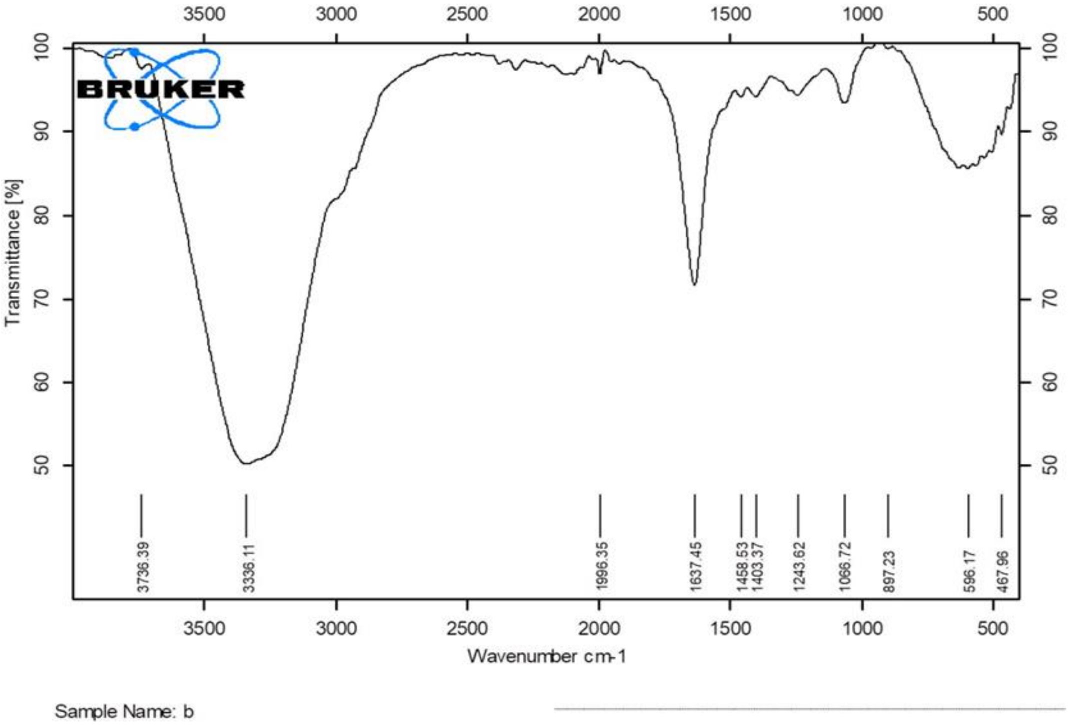

Fig.2. Spectrum of FTIR for Ag NPs

Energy Dispersive X-ray Spectroscopy analysis

The EDX spectrum confirms the elemental composition of the biosynthesized **Ag NPs**, with prominent peaks corresponding to silver (Ag) around 3 keV and oxygen (O) near 0.5 keV. The strong intensity of the Ag peak verifies the successful formation of silver-based nanostructures, while the O peak supports the presence of silver oxide rather than metallic silver alone. Additionally, a small carbon (C) peak may be observed, which is likely due to residual organic matter from the plant extract used in the green synthesis as shown in Figure (3) [12]

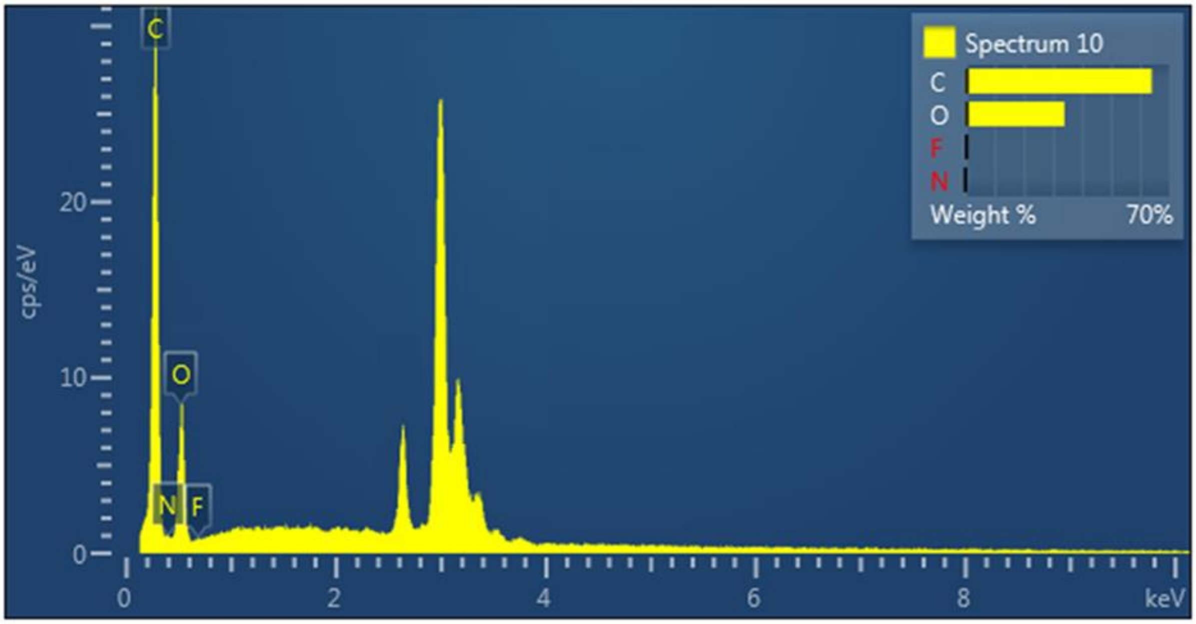

Fig.3. The EDX analysis AgNP.

FE-SEM analysis

The FE-SEM image at low magnification reveals a uniform distribution of Ag nanoparticles synthesized via green methods using plant extract. The particles appear predominantly spherical to quasi-spherical, with some degree of aggregation likely caused by the presence of residual phytochemicals acting as natural capping agents. Despite this slight clustering, individual particles are distinguishable, and their sizes range approximately between 40–80 nm. The smooth morphology and moderate agglomeration are consistent with biosynthesized metal oxide nanoparticles, where bioactive compounds such as flavonoids and phenolic acids influence both nucleation and growth during synthesis. (4A) Figure confirms that the green synthesis route successfully produced nanoscale particles with acceptable uniformity and dispersion At higher magnification, the FESEM analysis provides more detailed insight into the surface structure and particle interfaces of the Ag nanoparticles. The particles exhibit relatively smooth surfaces, but with some uneven edges and fused boundaries, which could indicate partial sintering during the drying phase. Additionally, the presence of fine organic layers surrounding the nanoparticles may be attributed to capping by plant-derived compounds, which are often retained after green synthesis and may serve as stabilizing agents. The high-resolution image demonstrates that the particles are within the nanoscale range and lack significant morphological defects, thereby supporting the successful biosynthesis of crystalline Ag nanostructures suitable for applications in catalysis, antimicrobial coatings, or drug delivery systems as shown in (4B) figure [14]

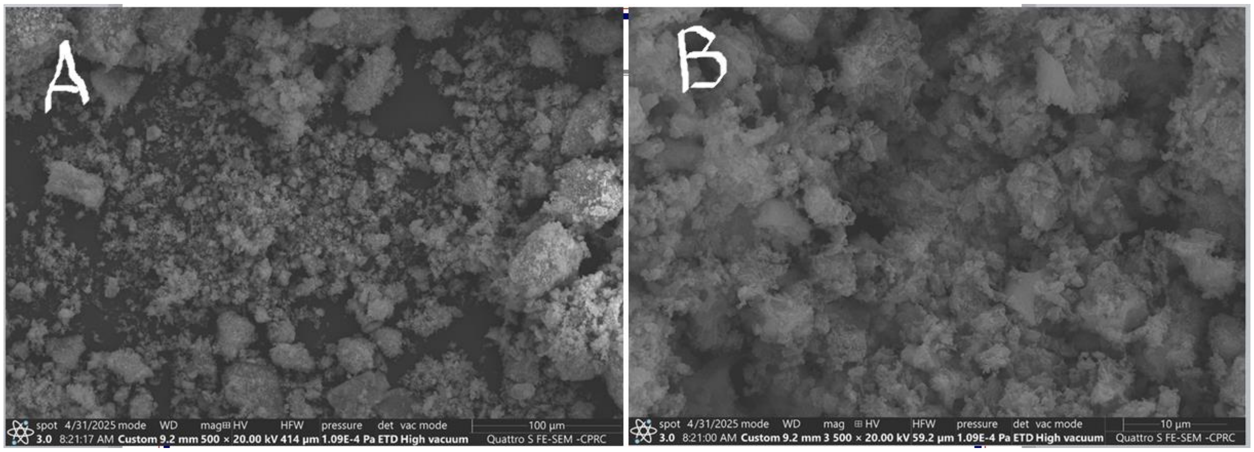

Fig.4.FE- SEM micrograph of AgNPs.

Optimization of urea biosensor

Effect electrolyte Concentration in Potentiometric System

Some salts are sometimes used as supporting electrolytes for buffer solutions in selective sensors. These salts, such as potassium chloride (KCl) or potassium nitrate (KNO3), are often added to increase the electrical conductivity and sensitivity of the biosensor. In this study, figure (5) shows the potential concentration versus the logarithmic concentration of the prepared urea, yielding a linear result

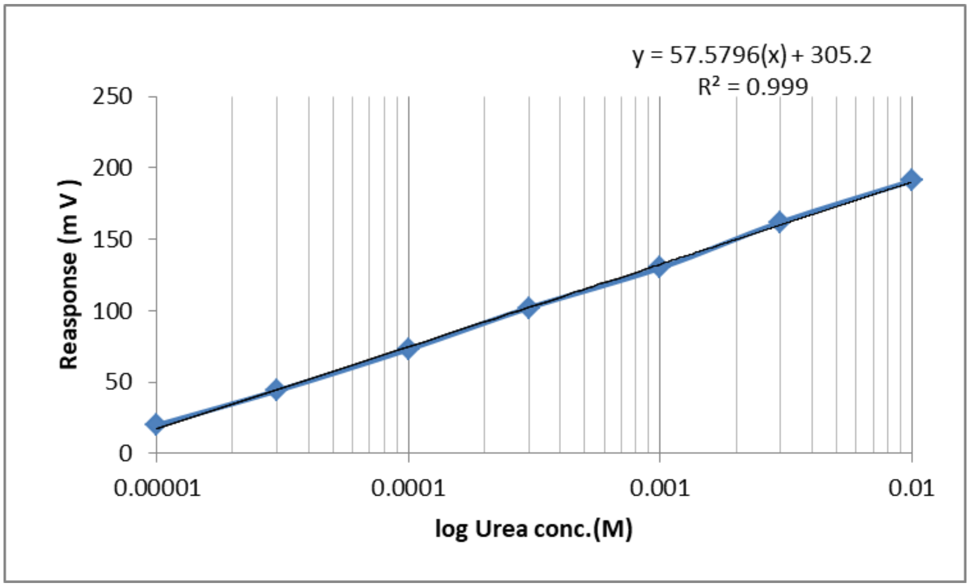

Fig.5. Response of the past electrode in potentiometric system of urea injection with (0.001M) KCl solution.

The optimal concentration that achieved the highest sensitivity for the paste electrodes was 0.001 M. The figure shows the result of enhancing the effect of potassium chloride using the pasted urea electrode, demonstrating that potassium chloride (KCl) acts as a protective layer, increasing the electrode's conductivity, improving its response and sensitivity, and preventing unwanted ionic interference. Its most important role is to support the electrodes [15]

Effect of Buffer Concentration on Biosensor Response

Studying and taking different concentrations of buffer increases the possibility of making the electrode with better and higher selectivity. Therefore, several concentrations of phosphate buffer were prepared, and these concentrations were tested on the prepared electrode as in the figure (6)

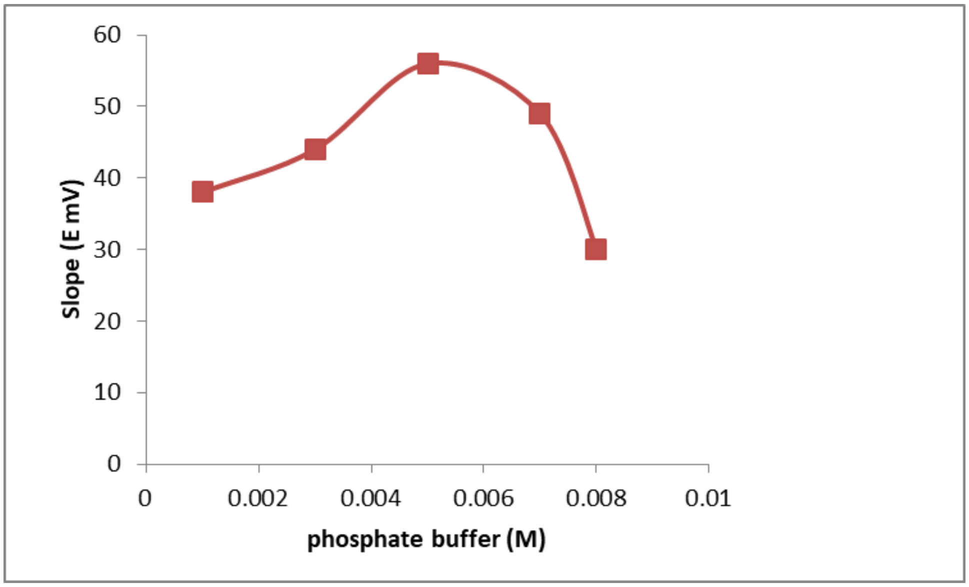

Fig.6. Effect of Buffer Concentration on biosensor.

In the figure above, the relationship between the slopes versus the buffer solution concentrations is plotted. The best concentration of phosphate buffer with the highest sensitivity was 0.005 M. There was an increase in the potential and sensitivity before this concentration, and a sharp decrease in the slopes of the biosensors after 0.005 M. At the same time, the sharp decrease in the response of the biosensor at phosphate buffer solution concentrations greater than 0.005 M was explained by the inhibition of the enzymatic reaction, while the lower phosphate buffer solution concentration gave the effect of a lower ionic concentration[15-17]

Effect of Phosphate Buffer pH

After ensuring the optimum concentration of the buffer solution for the paste electrode operating within an electrical circuit using a silver wire, the pH must be selected to ensure the electrode operates at the highest possible efficiency for the longest possible period. The electrode must also be stored in a suitable acidic medium. A Phosphate Buffer solution with a concentration of 0.005 M was tested over a different pH range (4.5-9.5). The pH values of these buffer solutions were tested on the paste electrode modified with nano-silver oxide to achieve better sensitivity. The study was conducted with urea concentrations of 0.001 M and in the presence of 0.001 M potassium chloride, and the curve shown in this figure was obtained as shown in this figure (7)

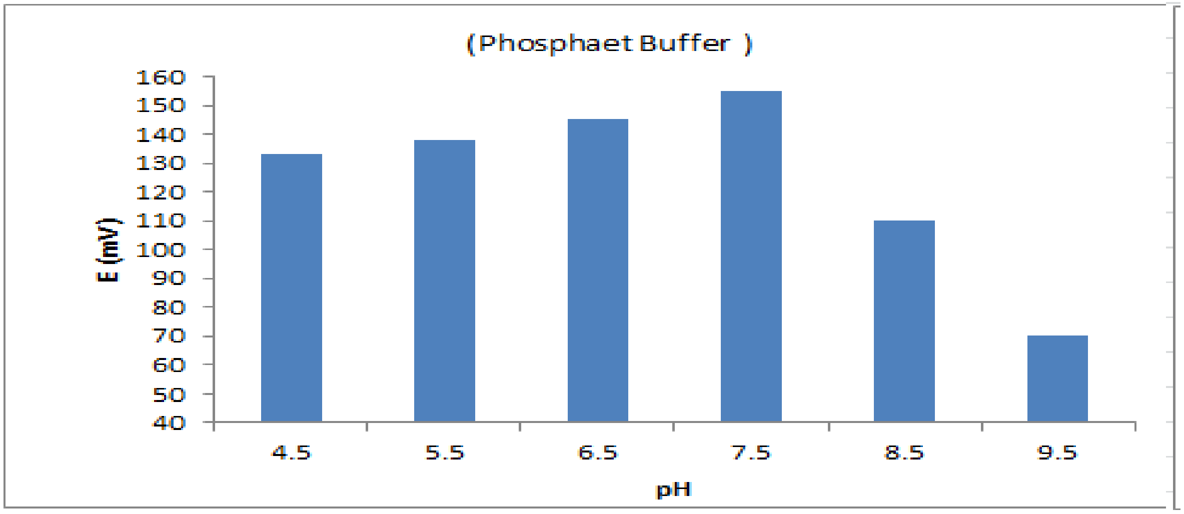

Fig.7. The effect of the reactant pH(4.5-9.5) with 0.001M of urea contains 0.001 M potassium chloride in Phosphate Buffer.

After it was confirmed that the best working medium acidity for the prepared electrode dough to study urea concentrations was pH (7.5) by giving the highest potential to the urea concentration of 0.001M figure, then the linear concentrations of the prepared urea were taken from 0.01 to 0.00001M to study the effect of pH (7.5) on the sensitivity and linearity. We note the occurrence of a shift in sensitivity that worked to increase the selectivity of the prepared electrode as shown in figure (8)[15],[18]

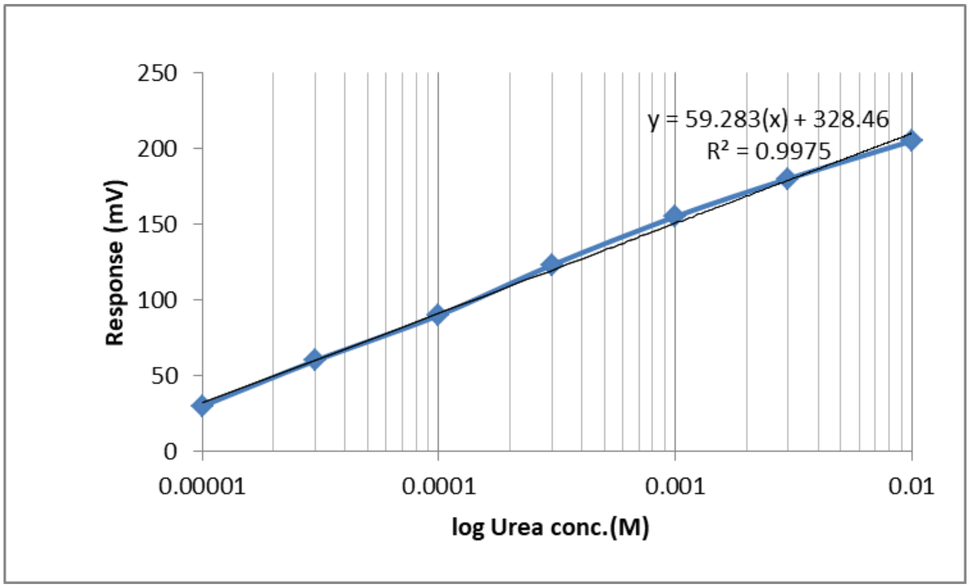

Fig.8. sensitivity of paste electrode with Phosphate Buffer.

The effect of temperature on the electrochemical response

It was investigated how temperature affected the dough's electrodes. At 40 °C, the response to urea peaked, and as the temperature rose above that, it declined. Enzymes are known to be temperature-dependent, meaning that their activity decreases with decreasing temperature. Up to the ideal temperature, increasing the medium's temperature causes an increase in activity. Due to the potential breakdown of the protein-structured enzyme molecules, the enzyme activity dropped (9) significantly at temperatures higher than the ideal one (in this case, 40 °C) Figure (9) provides a summary of how temperature affects the electrochemical response[18].

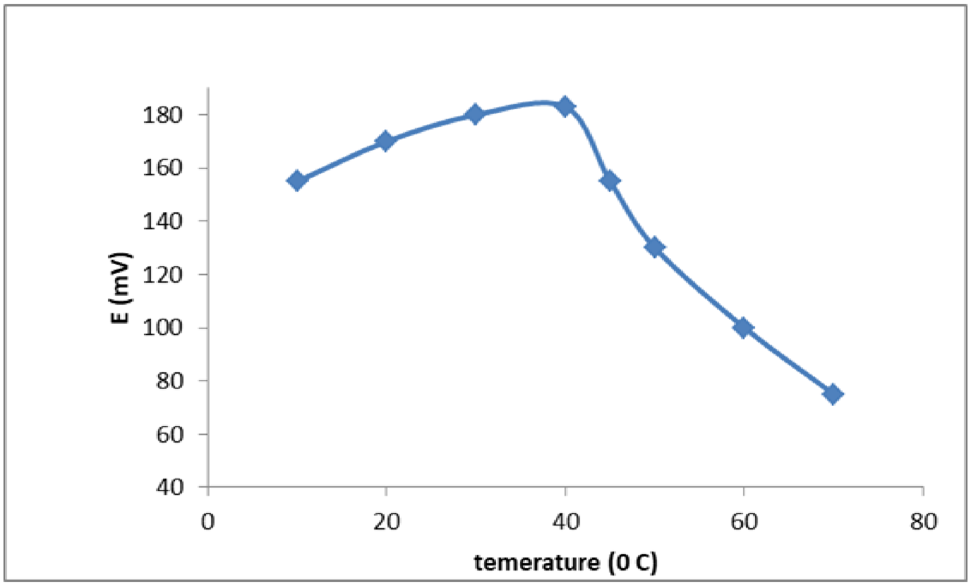

Fig.9. Effect of temperature on the past electrode.

Although high temperatures cause an increase in the prepared electrode potential, room-temperature operation is preferred for enzyme biosensors to prevent evaporation, electrode damage during measurement and enzyme degradation. Therefore, operating at room temperature maintains high electrode stability over the long term, as room temperature operation is constant to prevent enzyme degradation at elevated temperatures[9].

Evaluation of Biosensor Response5-

-Sensitivity, Dynamic Range, and Detection Limit

After testing the response of the prepared paste electrode to various conditions of electrolyte concentration, buffer concentration, appropriate pH, and final temperature, the results obtained for this electrode were recorded in the table(1) Below

Table 1: Sensitivity, Dynamic Range, and Detection Limit

type electrode	The Correlation Coefficient, R^2	Sensitivity	Linear Range (M)	Intercept
past electrode	**0.9975**	**59.283**	**0.01-0.00001**	**328.46**

While, the Detection Limit for the fourth electrode appeared to be lower according to the amount of linearity in it for the concentrations that were sensed by the electrode, which was greater and reached a value of 3.2* 10^{-5} M with Linear Range (0.01-0.00001) M Due to the high homogeneity between the prepared paste electrode components as a urea sensor and the silver oxide nanomaterial and urease enzyme activity, a wide range of urea concentration detection was achieved for this sensor system. and Relative standard deviation take to three measurement .RSD% (n=3)is from 0.58-1.1% to liner Urea concentrations The suggested sensor was able to evaluate urea in actual samples thanks to the measurements shown in the above. Because of its ease of use, mobility, and inexpensive manufacturing cost, the recently created enzyme sensor thus marks a substantial advancement in the field of urea detection[19].

Biosensor Response Time, Reproducibility, Stability, Selectivity and Application

Response Time

The response time of the urea biosensors was determined by recording the time elapsed to reach a stable potential value after the biosensor and the reference electrode were immersed in calibration solutions.The biosensor's response time was studied between 10 seconds and 100 seconds, with a 20-second interval as shown in figure (10)

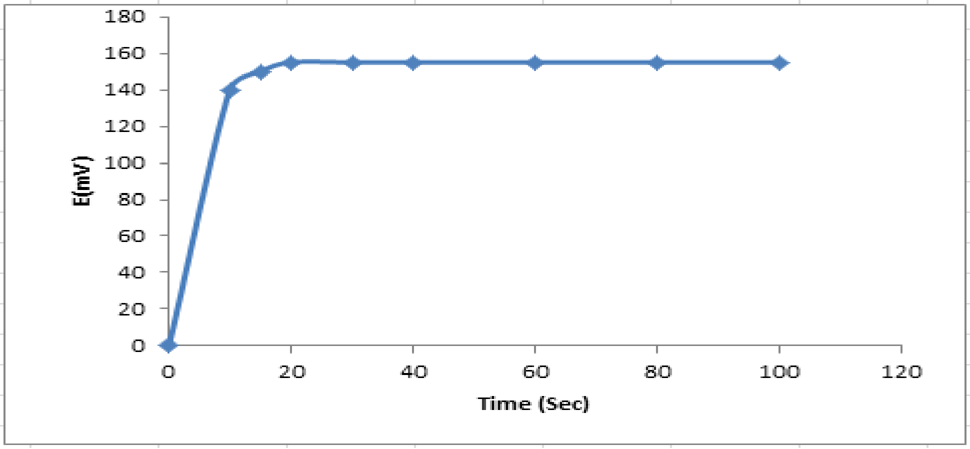

Fig.10. Response time of electrode of urea biosensor based on urease past electrode.

The results showed the response of obtaining a phosphate buffer solution with a pH of 7.5 with 0.005 M, 0.001 M potassium chloride, and 0.001 M urea The shorter and faster the response time of the prepared electrode, the better the selection and measurement, and it indicates the complete success of the electrode in the way it was prepared[20].

Biosensor Reproducibility

To evaluate the reproducibility and long-term stability of the proposed sensors, we independently fabricated five sensor electrodes under the same conditions, blending the urease enzyme into the silver oxide nanoparticles. Due to the homogeneity of the components with the enzyme in the presence of silver oxide, they can be reused multiple times with proper cleaning within two to three hours. The sensor response in a 0.001 M urea solution was highly reproducible up to five reuses, as shown in Figure (10). The relative standard deviation of the sensor electrode fabricated in standard urea solutions was less than 5%[9]

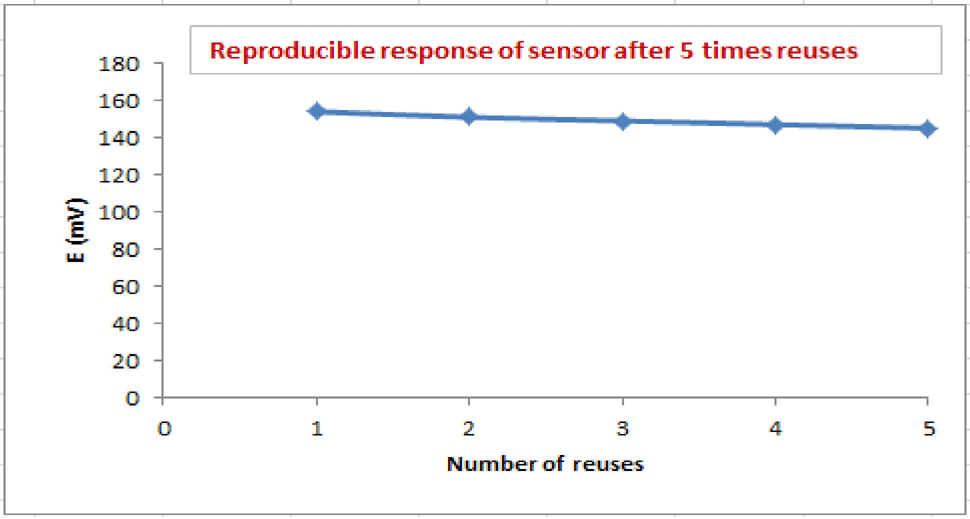

Fig.10. Biosensor Reproducibility.

Biosensor Stability

Stability is one of the most important properties of potentiometric urea biosensors. Nanomaterial-based sensors have demonstrated very high stability due to their high stability. These materials provide greater stability to biosensors with selective electrodes for pathological molecules, especially enzymes, which are selective for urea.In clinical trials, biosensor lifetime is a crucial metric for determining a biosensor's storage life and long-term measurement quality. At a linear concentration, lifetime was defined as the amount of storage time during which the relative sensitivity dropped by 10%. The urea biosensor electrode made from paste electrodes at the prepared urea concentrations was tested for sensitivity and stability by measuring potentials over a 10-day period. The results showed linearity and obvious sensitivity right away as shown in the figure (11).

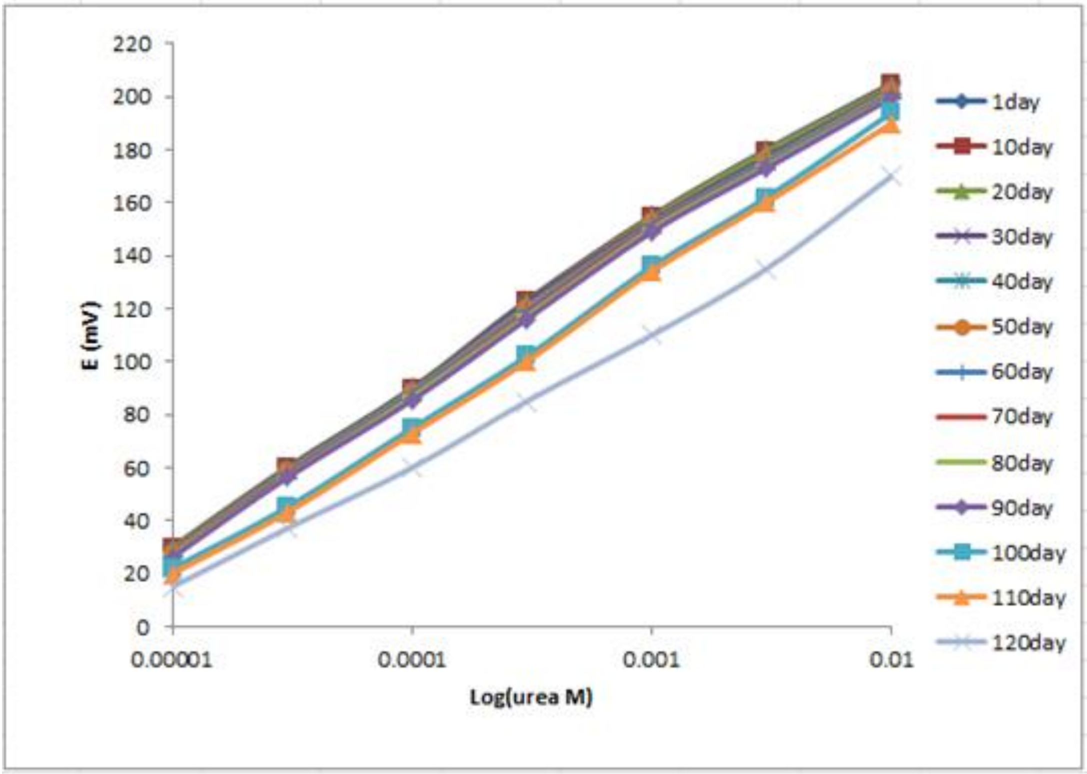

Fig.11. storage and stabilities electrode.

The biosensor's sensitivity measured to verify its lifetime. In this experiment, to test the long-term stability of the potentiometric urea biosensor, the sensor demonstrated high stability when measured on the same electrode every 10 days. Stability was approximately 90 days, or more than three months. After each measurement, the electrode was washed with distilled water two to three times, dried, and then stored at a low temperature in a refrigerator at 4°C. After 120 days, the prepared electrode completely collapsed, and readings became inaccurate due to the greatly reduced sensitivity across different concentrations [21].

The selectivity of urea sensors (interferences Study)

Interferences can occur for various reasons when studying the biosensor's response to urea. Some compounds may exhibit properties or structures similar to urea, which may cause the sensor to respond nonspecifically. In addition to interacting with the enzyme (urease), some compounds may modify the sensor's response by affecting the electrical conductivity or altering the signal response. Due to the sensor's low selectivity for urea alone, it may receive false signals or inaccurate responses if it interacts with other substances as well. To demonstrate the selectivity of the prepared urea sensor toward the target ion in the presence of competing ions (interfering ions), selectivity studies were performed. The biosensor's selectivity and stability were verified through output-response curves. Blood contains some potential interfering agents that may interfere with urea, such as ascorbic acid (AA), glucose (GL), uric acid (UR), lactic acid (LA) and cholesterol. These interfering agents were selected to confirm the selectivity of the prepared urea sensor toward the target urea molecule. For this purpose, a 0.001 M urea solution was prepared containing glucose (0.005 M), uric acid (0.00015 M), lactic acid (0.0055 M), ascorbic acid

(0.000005 M), and cholesterol (0.0052 M) as shown in Table (2). The amount of interference was calculated according to equation were examined

$$\text{Interference } (\%) = \frac{|V_i - V_u|}{V_u} \times 100$$

.

Where Vi and Vu are the peak voltage recorded for the mixed analyte (interfering substance + urea) and urea alone, respectively[22].

Table 2: Values of selectivity coefficient for different interfering past electrode.

S. No	Composition	Voltage (mV)	Difference	% interference
1	urea(0.001M)	155	155	0
2	urea(0.001M)+ glucose (0.005M)	155.8	0.8	0.51
3	urea(0.001M)+uricacid(0.00015 M)	157.3	2.3	1.48
4	urea(0.001M)+lacticacid(0.0055 M)	157	2	1.2
5	urea(0.001M)+ascorbicacid(0.000005 M)	155.5	0.5	0.32
6	urea(0.001M)+ cholesterol (0.0052 M)	154.1	1.1	0.70

From the above tables, there were no high readings with the potential difference between the added material and the pure material, as all materials recorded a percentage of less than 5%. This is evidence that the electrode is highly selective for urea only, and there are no interferences that are taken into consideration and do not cause distortion of the readings. Which indicates that they have negligible interference with the working electrode. Biosensors based on nanomaterials have shown excellent performance, including the lack of interfering effects. Electrical biosensors' sensitivity and selectivity can be enhanced by the physical and electrochemical characteristics of nanomaterials and their architectures. As a result, the development of low-cost electrodes based on nanomaterials has received more attention. Since the structure of the composite material used in chemical sensors' electrodes directly affects their performance, nanostructures are useful in the creation of electrochemical sensors. Significant growth acceleration has been shown by these sensors, which may result in the creation of new technologies[23, 24].

Application of the Improved Urea Biosensor

The study included blood samples from 97 individuals, obtained from Al-Imamain Al-Kadhimiya Hospital and Nuclear Medicine in Baghdad, Iraq, who work with ionizing radiation equipment. Blood was separated, serum samples were taken from the individuals, and their urea concentration was determined using the in vitro clinical method and the current biosensor. A set of samples with different urea concentrations was selected and determined using the in vitro method to monitor the potential differences determined by the prepared electrode. The method involved adding 0.1 ml of serum to 10 ml of reaction solution (0.001 M phosphate buffer, pH 7.5). The urea concentration was derived from a standard curve between the urea concentration and the biosensor response in volts, prepared under optimized conditions[25]

Comparison study

Accuracy and Precision

Analytical Recovery (accuracy)

Analytical Recovery is a test used to determine the accuracy and efficiency of a device or method used to measure a specific substance (such as blood urea). This can be detected and determined using a prepared electrode. We take a sample (for example, blood serum) and measure the amount of urea using the quantum method. Then, we measured the concentration using the prepared analytical method (biosensor). We add a known amount of the substance we want to measure (such as urea), then use the device or sensor to measure the amount of urea. If the device is accurate, it will give a number very close to the amount we added. The calculation is as follows: A serum sample was taken and the urea content was measured using the method and found to be 15 mg/dL (0.0024 M). The same sample was also measured using the biosensor method to confirm the concentration after add phosphat buffer and found to be (0.0025 M). Known concentrations of prepared urea were added at concentrations of (0.0025 - 0.0053 - 0.0091), and the resulting concentration after addition to the prepared electrode was calculated by calculating the potential and finding the concentration using the Nernst equation. All data are shown in the table (3).

Table 3: Analytical Recovery (accuracy) of past elctrode

Added urea	Found urea	Recovery (%)
0.0025 M	**0.0052 M**	**108%**
0.0053 M	**0.0078 M**	**100%**
0.0091 M	**0.0119M**	**103%**

All results gave recoveries between 100% and 108%. This indicates that the sensor was very accurate and gave readings very close to reality. If the percentage is between 95% and 110%, this is considered evidence of the accuracy of the method[25]

Precision

Precision was previously explained through the terms Biosensor Reproducibility and Repeatability and finding the average and standard deviation to calculate the average standard deviation of the electrodes prepared from the paste containing nano silver ochre with the enzyme urease to measure urea. It resulted that whenever the standard deviation of the electrodes prepared from the paste was less than 10% electrodes are compatible with each other and the precision of the method / electrode is satisfactory

Correlation

To determine the accuracy of the present biosensor, urea values (mg/dL) in 6 searm samples were determined by the present biosensor method and compared with those obtained by the reference method (Enzymic kit method) used in hospitals. The values obtained by both the methods were correlated using regression equation. The regression plot Figure(12) between the two methods was drawn and the correlation coefficient was calculated showing a good correlation (R2=0.9999) between the urea values measured by the reference method and present biosensor. Compared to reference methods, the present bio sensing method for determination of urea in serum is more simple, sensitive, specific and rapid[25].

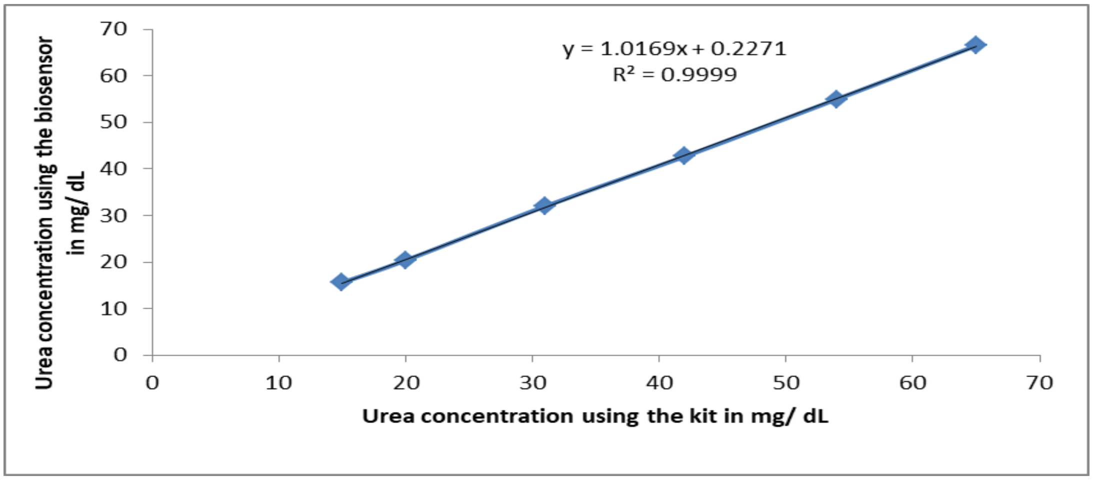

Fig.12. Correlation between measured by standard enzymic kit method and biosensor based on urease membrane.

Some studies that depend on estimating urea using potencimetry were collected and placed in a table (4) for comparison with the current study

Table 4. Comparison of different potentiometric urea biosensors with presented study.

Support	sensitivity	**Range**	**LOD**	Response	Stability	Reuse	Ref.
urease nanoparticles	23 mV/decade	(0.002-0.08 mM)	1µmol/L	20-42 sec	180 days	8-9 times	[25]
ZnO nanowires	52.8 mV/decade	0.1 mM to 100 mM	0.1 mM	4 sec	three weeks	8times	[9]
poly(2-hydroxyethyl methacrylate-glycidyl methacrylate) nanoparticles	-	0.01 mM-500 mM	0.77 µM	30 sec	40day	170	[18]
zinc oxide	187.5 µV ppm−1 cm−2	20 ppm to 500 ppm	29.84 ppm	3 min	eight weak		[22]
Past(Ag_2O)	59.28	0.01-0.00001M	$3.2*10^{-5}$	30sec	90day	5time	The presented study

Conclusion

The electrodes were prepared by adding nanomaterial's and characterized by several techniques. They were improved by adding potassium chloride electrolyte and choosing the appropriate concentration and pH of phosphate buffer and stabilizing the measurements at room temperature. The urea-selective electrolyte biosensor gave excellent analytical results, including sensitivity, high selectivity, wide working range, low detection level, good repeatability, and high storage stability for months, which makes its application on samples better and more widely used in the future

Author's Declaration

- We hereby confirm that all the Figures and Tables in the manuscript are original and have been created by us.
- We have obtained ethical clearance for our study from the local ethical committee at [Al-Nahrain University]. This approval underscores our commitment to ethical research practices and the well-being of our participants.

- Ethical Clearance: The project was approved by the local ethical committee at [Al-Nahrain University], ensuring adherence to ethical standards and the protection of participants' rights and welfare.

Author's Contribution Statement

[First Author]: Contributed to the conception and design of the study, conducted some experiments, data rearrangement and drafted the initial manuscript.

[Second Author]: conducted some experiments, collection, conducted some characteristics of the products and wrote this research article.

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