A REVIEW: ESTIMATION OF URIC ACID BY USING DIFFERENT METHODS OF ELECTROCHEMICAL BIOSENSOR

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Abstract- **Uric acid analysis is extremely important in conditions like gout prognosis, diagnosis and treatment. The techniques used for estimation of uric acid shows lack specificity or exhibit poor performance, and thus could not meet the need for uric acid monitoring. This review article includes information for the measurement of uric acid based on different methods of electrochemical biosensor i.e., Point of care testing, Nitrogen doped graphene, thin film and Amperometry biosensor etc. And comparisons with other methods, the electrochemical method has a high sensitivity and fast detection. Thus, this review article provides information about the different types of diseases, treatment and biosensors used for estimation of uric acid in biological fluids i.e., urine and serum samples.**

Keywords: **Uric acid, Electrochemical biosensor, Point-of-care, Nitrogen doped graphene, thin film, Amperometry biosensor.**

I. INTRODUCTION:

Introduction of uric acid:

Definition of uric acid [1]:

Uric acid is a heterocyclic compound of carbon, nitrogen, oxygen, and hydrogen with the formula **$C_5H_4N_4O_3$.**It forms ions and salts known as urates and acid urates, such as ammonium acid urate.

Uric acid is a product of the metabolic breakdown of purine nucleotide, and it is a normal component of urine.

FORMULA: **$C_5H_4N_4O_3$**.

Molar mass: **168.1103 g/mol**

Soluble in: **Water**

Chemical structure of uric acid [1]:

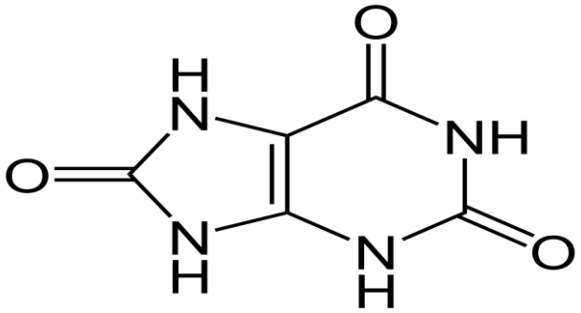

Fig. Structure of uric acid

properties of uric acid [1]:

Table. Properties of uric acid

PROPERTIES	
Chemical formula	$C_5H_4N_4O_3$
Molar mass	168.112 g·mol^{-1}
Appearance	White crystals
Melting point	300 °C (572 °F; 573 K)
Solubility in water	6 mg/100 mL (at 20 °C)

log P	−1.107
Acidity (pK_a)	5.6
Basicity (pK_b)	8.4
Magnetic susceptibility (χ)	−6.62×10−5 cm3 mol−1

Levels of uric acid [2]:

Table. Levels of uric acid

Uric acid level	Males	Females
Low	Below 2.5 mg/dl	Below 1.5 mg/dl
Normal	2.5-7.0 mg/dl	1.5-6.0 mg/dl
High	Above 7.0 mg/dl	Above 6.0 mg/dl

Diseases related to high and low level of uric acid [3]:

Uric acid is a waste product found in blood. It's created when the body breaks down chemicals called purines. Most uric acid dissolves in the blood, passes through the kidneys and leaves the body in urine. Food and drinks high in purines also increase the level of uric acid.

In body uric acid level increases this condition called Hyperuricemia. Hyperuricemia can occur due to formed crystals of uric acid in body. These crystals can settle in the joints and cause gout, a form of arthritis that can be very painful. They can also settle in the kidneys and form kidney stones.

If untreated, high uric acid levels may eventually lead to crystal deposited in joints, permanent damage of bone, joint and tissue damage, kidney disease and heart disease. Research has also shown a link between high uric acid levels and type 2 diabetes, high blood pressure, and fatty liver disease.

High level of uric acid [1] [4]:

Hyperuricemia (high levels of uric acid), which induces gout

- **Gout,** sometimes called gouty arthritis, occurs in about 20 percent of people with Hyperuricemia. A rapid drop in uric acid levels can also trigger gout. Gout can appear as isolated attacks, or flares. Some people experience chronic gout, which involves a number attacks occurring over short periods of time. Gout can affect any joint in your body, but flares often first appear in your large toe. Feet, ankles, knees, and elbows are also common sites of gout. Gout attacks tend to occur suddenly, often at night. The attacks peak in intensity in about 12 to 14 hours. Even untreated, attacks of gout usually subside within two weeks.
- **Symptoms of gout may include**:
- severe pain in your joints
- joint stiffness
- difficulty moving affected joints
- redness and swelling
- misshapen joints
- **Tumor lysis syndrome:**

Tumor lysis syndrome, an emergency condition that may occur from blood cancers, produces high uric acid levels in blood when tumor cells release their contents into the blood, either spontaneously or following chemotherapy Tumor lysis syndrome may lead to acute kidney injury when uric acid crystals are deposited in the kidneys. Treatment includes hyperhydration to dilute and excrete uric acid via urine, Ras uricase to reduce levels of poorly soluble uric acid in blood, or allopurinol to inhibit purine catabolism from adding to uric acid levels.

- **Lesch–Nyhan syndrome:**

Lesch–Nyhan syndrome, a rare inherited disorder, is also associated with high serum uric acid levels. Spasticity, involuntary movement, and cognitive retardation as well as manifestations of gout are seen in this syndrome.

- **Cardiovascular disease:**

Hyperuricemia may increase risk factors for cardiovascular disease.

- **Types 2 diabetes:**

Hyperuricemia may be a consequence of insulin resistance in diabetes rather than its precursor. It's showed high serum uric acid was associated with higher risk of type 2 diabetes independent of obesity, dyslipidemia, and hypertension. Hyperuricemia is associated with components of metabolic syndrome, including in children.

- **Kidney stone:**

Uric acid crystals can cause a buildup of stones in your kidneys. Often, the stones are small and are passed in your urine. Sometimes, they can become too large to pass and block parts of your urinary tract.

- Symptoms of kidney stones include:

- pain or aching in your lower back, side, abdomen, or groin
- nausea
- increased urge to urinate
- pain when urinating
- difficulty urinating
- blood in your urine
- foul-smelling urine
- If you also have a kidney infection, you may experience fever or chills.

Low level of uric acid [1]:

Low uric acid (hypouricemia) can have numerous causes. Low dietary zinc intakes cause lower uric acid levels. This effect can be even more pronounced in women taking oral contraceptive medication Sevelamer, a drug indicated for prevention of hyperphosphatasemia in people with chronic kidney failure, can significantly reduce serum uric acid.

- **Multiple sclerosis [1]:** Meta-analysis of 10 case-control studies found that the serum uric acid levels of patients with multiple sclerosis were significantly lower compared to those of healthy controls, possibly indicating a diagnostic biomarker for multiple sclerosis.
- **Normalizing low uric acid [1]:**

Correcting low or deficient zinc levels can help elevate serum uric acid.

- **Alzheimer's disease [8]:**

If uric acid level is extremely high or low then risk of Alzheimer's disease occurs.

- **Parkinson's disease [9]:**

Uric acid (UA) is a potent endogenous antioxidant, resulting from purine metabolism. It is responsible for about half of the antioxidant capacity of the plasma. Increasing evidence suggests that lower serum UA levels are associated with an increased risk of developing PD and with faster disease progression.

- **Xanthen stones [10]:**

A rare xanthenes dehydrogenase/oxidase enzyme and is characterized by very low (or undetectable) concentrations of uric acid in blood and urine and very high concentration of xanthenes in urine, leading to urolithiasis.

Treatment for the high and low level of uric acid [5]:

High uric acid level: Below are the home remedies to help you know how to control uric acid levels in your body.

- **Limit purine-rich food**

Limiting the intake of foods highly rich in purine can reduce uric acid levels in the blood.

- **Avoid alcohol**

Reduce alcoholic beverages, increase your water consumption. This can help your body get rid of excess uric acid by diluting your urine. Alcoholic drinks such as beer are also high in purines.

- **Lose weight**

Uric acid production is higher in fat cells than in muscle cells. Furthermore, gaining weight makes it more difficult for your kidneys to filter out uric acid. Losing weight drastically can have a negative impact on the levels. Consult your nutritionist about weight loss plans and healthy. Based on your body type, your doctor can recommend a healthy weight target.

- **Increase the intake of dietary fibre**

High-fibre diets are frequently prescribed to people who are dealing with uric acid disorders. Wholegrain foods are high in fibre. The fibrous components absorb and control all of the excess uric acid in the bloodstream.

Low uric acid level [7]:

- Uric acid levels may be high if you eat foods high in purines. These include organ meats, dried beans and peas, and fish such as anchovies, herring, sardines, and mackerel. High levels can also be caused by a low-salt diet.

Different methods for the measurement of uric acid [11]:

Two methods are widely utilized to quantify uric acid,

A **colorimetric** method depends on the reduction of a chromogen such as sodium tungstate by uric acid to produce a measurable color change. This technique has been commonly employed in automated hospital screening (SMA systems). The method measures materials other than urate, such as ascorbic acid. Colorimetric determinations are generally considered an overestimation of true uric acid levels, and the normal range is usually 1 mg/dl higher than the more specific enzymatic techniques.

Enzymatic determination of uric acid results from the specific oxidation of uric acid by uricase, which converts its substrate to allantoin. The differential absorbance of these substances at 293 nm allows quantification.

$$\text{Allantoin} + H_2O_2 + CO_2 \longrightarrow \text{Uric acid} + 2H_2O + O_2$$

(Nonabsorbing at 293 nm) (Absorbs at 293 nm)

Although traditionally a more expensive technique, uricase methods are currently available. And comparable costs and are gradually replacing the less specific colorimetric method.

INTRODUCTION OF ELECTROCHEMICAL BIOSENSOR [14]:

- Biosensors are analytical devices that interpret biological information into a quantifiable signal. Biosensors differ from other popular analytical systems, such as high-performance liquid chromatography (HPLC), in that they are self-contained integrated devices. However, biosensors can be incorporated into larger analytical systems. There, they act as a receptor device that can selectively collect quantitative or semi-quantitative information.
- Biosensors are made of five main components: a bioreceptor, an interface, a transducer element, computer software, and a user interface. The bioreceptor binds to the analyte selectively, and the interface is where a chosen biological event occurs that gives rise to the signal that is measured. The signal is measured by the transducer element. The transducer element, which in the case of electrochemical biosensors is an electrochemical transducer, turns the transducer signal into an electronic signal and amplifies it. The computer software converts the electronic signal into a physical parameter that can meaningfully be interpreted. This is then presented through the user interface to the operation.
- Electrochemical biosensors are a class of biosensors that function using an electrochemical transducer. They can detect biological materials such as enzymes, whole cells, specific ligands, and tissues, but also non biological matrixes.

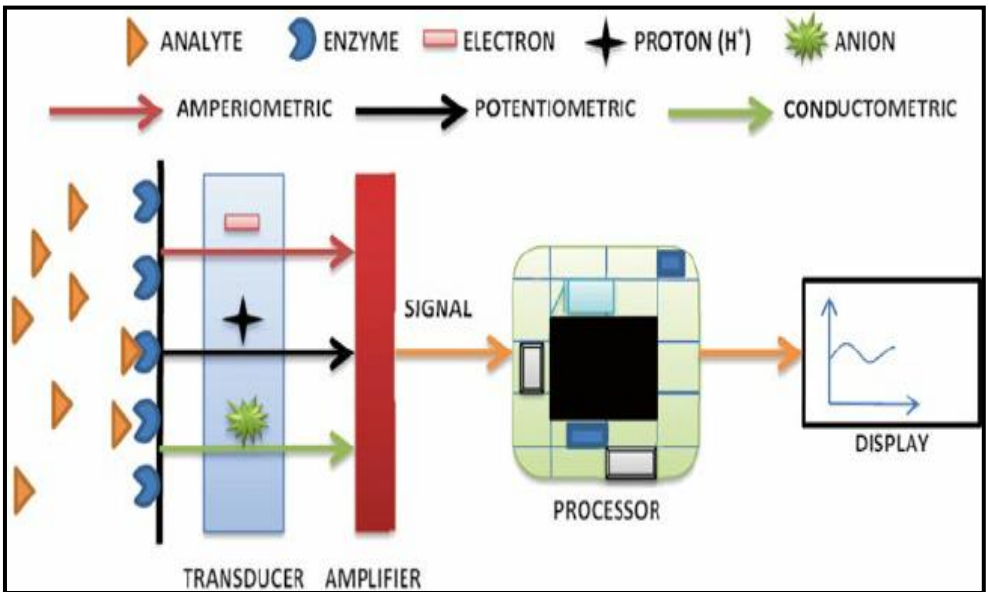

Fig. Schematic diagram of electrochemical biosensor

Definition [12]:

- An electrochemical biosensor is a self-contained integrated device, which is capable of providing specific quantitative or semi-quantitative analytical information using a biological recognition element (biochemical receptor) which is retained in direct spatial contact with an electrochemical transduction element.

Types of electrochemical biosensors [13]:

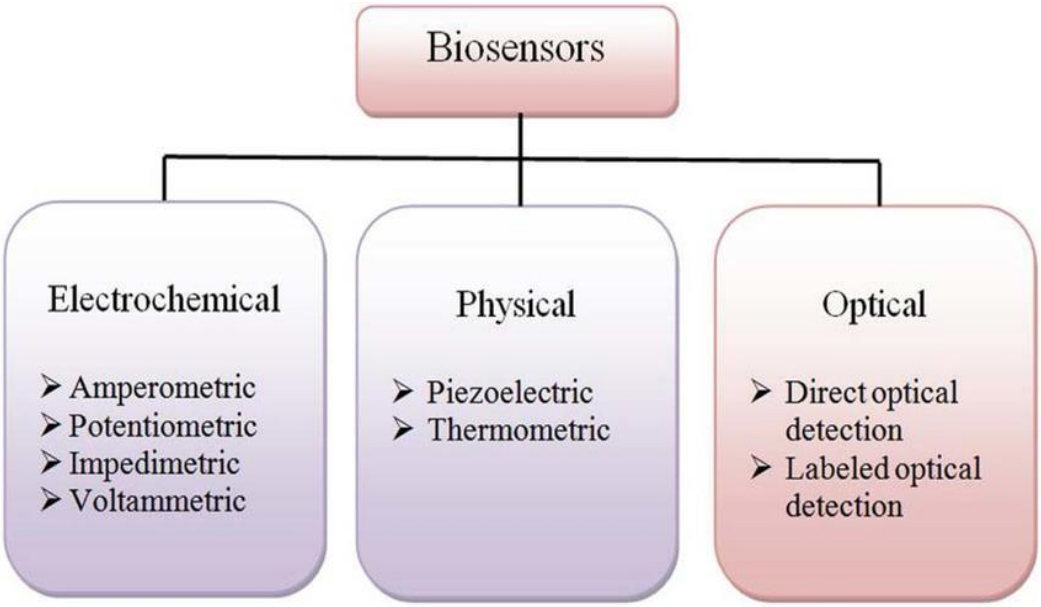

Fig. Types of biosensors

- A number of different types of electrochemical biosensors exist depending on their method for ensuring biological selectivity or their signal transduction method, or a combination of the two. The receptor can be selective for a biocatalytic event such as a reaction that is catalyzed by macromolecules, including enzymes, or for a specific bio affinity, such as the interaction between an analyte and a macromolecule, independent of their biological environment.
- The detection methods for electrochemical transducers are diverse [14].

I. **Amperometry**, which is based on measuring the electroactive species electrochemical oxidation or reduction current. The current relates directly to the concentration of the electroactive species[14].

II. **Conductometric devices** measure the ability of an analyte *(*e.g., electrolyte solutions) or a medium *(*e.g., nanowires) to conduct an electrical current between electrodes or reference nodes. Although Conductometric devices can be considered as a subset of impedimetric devices, techniques for measuring capacitance changes are reviewed later in combination with electrochemical impedance spectroscopy. Conductometric devices have been strongly associated with enzymes, where the ionic strength, and thus the conductivity, of a solution between two electrodes changes as a result of an enzymatic reaction. Thus, Conductometric devices can be used to study enzymatic reactions that produce changes in the concentration of charged species in a solution. The variable ionic background of clinical samples and the requirement to measure small conductivity changes in media of high ionic strength limit the applicability of such enzyme-based Conductometric devices for biosensing. Another approach is to directly monitor the changes in conductance of an electrode as a result of the immobilization of *e.g.*, enzymes, complementary antibody-antigen pairs, etc. onto the electrode surface [13].

III. **Potentiometry** is a detection method that involves the potential difference of an indicator electrode and a reference electrode when no current flows between them. These are most commonly pH electrodes but can be ion-selective electrodes. electrochemical biosensors can measure analytes by the surface charge using field-effect transistors. These can determine ion concentrations by attaching an ion-selective membrane to a transistor, which becomes a biosensor when coupled with a biocatalytic or bio affinity layer [14].

Advantages and disadvantages of biosensors [14]:

- Electrochemical biosensors have distinct advantages, such as lacking the high complexity of the sensor setup and the high cost of other biosensors. Instead, electrochemical biosensors are associated with the cheap production of microelectronic circuits and a user-friendly interface with conventional electronic processing and results.
- Electrochemical biosensors are also robust, easy to miniaturize, and offer broad detection limits even when only small volumes of analyte are present. Furthermore, electrochemical biosensors can be used to analyze biofluids with high turbidity and optically absorbing and fluorescing compounds.
- Electrochemical biosensors have certain features that hinder further developments.
- Because of this, nanotechnology has had increased applications in electrochemical biosensing by being used to minimize elements of electrochemical sensor into sizes that increase the signal-to-noise ratio. This is particularly important for processes that are intended to occur at the device's interface and can be used to discover methods to use several enzymatic labels to increase the intensity of the event signal.

Applications of electrochemical biosensors [14]:

- One of electrochemical biosensors uses is to directly monitor analytes or the biological activity related to the analyte (production or consumption). Through this, the biosensor can monitor the activities of living cells or enzymes.
- Indirect monitoring can be done to analyze organic pesticides or inorganic substances that interfere with the biocatalytic properties of the sensor. This involves monitoring of heavy metals, fluoride, cyanide, and others that have high importance in industry and environment. However, these are often irreversible, and therefore often provide less exact analyte concentration measurements.

II. METHODS FOR THE DETERMINATION OF URIC ACID BY USING ELECTROCHEMICAL BIOSENSOR:

3.1 Point of care testing methodology
3.2 Super-Align carbon nano tube array
3.3 Thin film base nano biosensor
3.4 Nano tube cuprous oxide – ferrocene-uricase modified glassy carbon electrode
3.5 Composites of bimetallic platinum-cobalt alloy nano particles & the reduce graphene oxide
3.6 β – cyclodextrin – selenium doped carbon quantum dots modified glassy carbon electrode
3.7 Nitrogen doped graphene
3.8 Polyaniline / Prussian-blue – based amperometry biosensor
3.9 Mesoporous silica (MCM-41) for enzyme immobilization
3.10 Portable electrochemical biosensor
3.11 Grapheme quantum dots as a new substrate for immobilization of uric oxidase
3.12 Nano –Au / DNA /nano – Au / Poly (SFR) composite
3.13 Uricase immobilized on ZnO nanowires
3.14 Mesoporous CO_3O_4 nanosheets
3.15 Amperometry biosensor

3.1. Point of care testing [16]:

- Point-of-care technology is an important method in clinical testing in the future, which can achieve the purpose of rapid analysis.
- In this work, electrochemical point of care sensor for uric acid by surface modification of a screen-printed electrode
- Copper nanowires were used as electrode modifiers to achieve high-performance electrochemical oxidation of uric acid.
- This electrochemical sensor can achieve linear detection of uric acid in the range of 10μM to 2 mM.
- The detection limit of the sensor was calculated to be 2 µM.
- It has also been used successfully to test urine and serum samples from healthy and gout patients.

3.2. Super align carbon nanotube array (SACNT) [17]:

- Novel electrochemical biosensor based on 3D Super align carbon nanotube array to facilitate point of care uric acid monitoring.
- The working electrode of the biosensor is composed of an orderly 3D SACNT array immobilized with uricase through a precipitation & crosslinking procedure
- The biosensor possesses higher enzyme density, significantly larger contact area with reactants & could maintain the intact SACNT structure & its excellent conductivity after modification & high sensitivity of 518.8 µ A/(mM.cm$^{2)}$, wide linear range of 100 to 1000 µM & low limit of detection of 1µM for uric acid.
- The obtained results in serum samples had no significant difference compared with those obtained using the FDA approved electrochemical analyzer (paired T-test, $p>0.05$).

3.3. Thin film base nano biosensor [18]:

- This includes sensing principles, electrode types and various thin film matrices.
- The theoretical and experimental results suggest that the thin film-based sensor is suitable for highly sensitive detection of low concentration bio molecules
- This used for early diagnosis and screening of arthritis, kidney diseases and other cardiovascular diseases.
- This method concludes that enzyme immobilization and surface fictionalization methods were used to increase sensitivity of the biosensor and new combination of polymer nano-composites with metal oxide thin films results in increased efficiency due to its high biocompatibility.

3.4. Nano tube cuprous oxide- ferrocene-uricase modified glassy carbon electrode [19]:

- A uric acid electrochemical biosensor was constructed using ferrocene decorated cuprous oxide enhanced electro-active characteristics and covalently immobilized with uricase on glassy carbon electrode.
- Differential pulse voltammetry studies revealed rapid response of fabricated electrode uricase /ferrocene/cuprous oxide/glassy carbon electrode.
- Towards uric acid in a wide concentration range of 0.1–1,000 µM with a sensitivity of 1.900 µA mM^{-1} cm^{-2} and very low detection limit of 0.0596 µM.
- A very low magnitude Michaelis–Menten constant (Km) Value was evaluated as 34.7351 µM which indicated the chemical attraction of the enzyme towards the UA was much higher.
- The developed biosensor was successfully applied to detect uric acid in human urine samples.
- Reproducibility and stability studies demonstrated the fabricated uricase /ferrocene/cuprous oxide/glassy carbon electrode biosensor had high reproducibility with an RSD of 2.8% and good reusability with an RSD of 3.2%.
- Specificity studies results showed the fabricated biosensor had strong anti-interference ability.
- The improved sensor performance was attributed to the synergistic electronic properties of cuprous oxide and ferrocene that provided enhances electrocatalytic activity and electron transfer.

3.5. Composites of bimetallic platinum-cobalt alloy nano particles & the reduce grapheme oxide [20]:

- The ultimate aim of this study is to produce a composite of bimetallic platinum- cobalt nanoparticles and reduce grapheme oxide-based biosensor for the detection of uric acid.
- Platinum-cobalt nanoparticles and reduce grapheme oxide was synthesized using a microwave-assisted technique and utilized for the production of a highly sensitive and stable electrochemical biosensor.
- The prepared platinum-cobalt nanoparticles and reduce grapheme oxide- based biosensor showed high electrochemical activity, a broad linear response, high sensitivity, and acceptable limit of detection values for individual and simultaneous determination of uric acid under optimized conditions.
- The linear range of platinum-cobalt nanoparticles and reduce grapheme oxide was found to be 170–200, 35–1500 and 5–800 µM for uric acid.
- The detection limit of the prepared composite was calculated as 0.172 µM for uric acid.
- In the field of electrochemical biosensors, platinum-cobalt nanoparticles and reduce grapheme oxide- based sensor is highly promising due to its superior sensitivity and good selectivity properties**.**

3.6.β– cyclodextrin – selenium doped carbon quantum dots modified glassy carbon electrode [21]:

- The β-cyclodextrin/selenium-carbon quantum dots composite was characterized by the transmission electron microscopy and Fourier transform infrared spectroscopy.
- The electrochemical behavior of uric acid on the modified electrode was investigated by cyclic voltammetry, electrochemical impedance spectroscopy and differential pulse voltammetry.
- The oxidation peak was found in concentration range of uric acid 10 ≈1000µM with the limit of detection 0.03µM.
- This biosensor exhibited prominent selectivity, stability and reproducibility and it was use for determination of uric acid in human serum and urine sample with satisfactory results.

- This sensor exhibited wider linear range and lower detection limit.

3.7 . Nitrogen doped graphene [22]:

- Nitrogen doped graphene was prepared by thermally annealing graphite oxide and melamine mixture.
- After characterization by atomic force microscopy and X-ray photoelectron spectroscopy etc., the electrochemical sensor based on nitrogen graphene was constructed to simultaneously determine small biomolecules such as ascorbic acid, dopamine and uric acid.
- Due to its unique structure and properties originating from nitrogen doping, nitrogen graphene shows highly electrocatalytic activity towards the oxidation of ascorbic acid, dopamine and uric acid.
- The electrochemical sensor shows a wide linear response for ascorbic acid, dopamine and uric acid in the concentration range of 5.0×10^{-6} to 1.3×10^{-3} M, 5.0×10^{-7} to 1.7×10^{-4}M and 1.0×10^{-7} to 2.0×10^{-5}M with detection limit of 2.2×10^{-6} M, 2.5×10^{-7}M and 4.5×10^{-8}M at S/N = 3, respectively.
- These results demonstrate that NG is a promising candidate of advanced electrode material in electrochemical sensing and other electrocatalytic applications.

3.8. Polyaniline/Prussian-blue–based amperometry biosensor [23]:

- Polyaniline/Prussian blue composite was electrochemically deposited on a platinum electrode.
- The properties of the Polyaniline/Prussian-blue–based modified electrode were exploited in terms of its response toward H_2O_2 sensing by using cyclic voltammetry, and were found to be more effectual than the PANI-modified electrode in the low-potential range.
- The Polyaniline/Prussian-blue–based modified electrode was further used for the bioanalysis of uric acid by immobilization of the enzyme uricase.
- The biosensor gave a linear response for uric acid in the Concentration range 10–160 mm with a sensitivity of 160 $mAmm^{-1}cm^{-2.}$
- The main advantage of the Polyaniline/Prussian-blue–based composite electrode is its low working potential (0 V vs. Ag/AgCl) forth estimation of uric acid, which enables the elimination of plausible interfering effects caused by analytes present in real Samples.
- The biosensor was selective to uric acid and did not respond to analytes such as ascorbic acid, glucose, and urea.
- The sensor electrode retained its selectivity and sensitivity for seven days if stored at -18ºc.
- The biosensor also displayed satisfactory performance for the detection of uric acid inhuman blood serum samples.

3.9. Mesoporous silica (mcm-41) for enzyme immobilization [24]:

- The mesoporous material was immobilized by nafion (5%ethanolic solution) assisted adsorption on a glassy electrode, which was used as working electrode.
- The biosensor configuration allowed a fast, sensitive and stable electrochemical detection of hydrogen peroxide generated by the enzyme reaction.
- Amperometry in stirred solutions at -100mV provided a linear calibration plot for uric acid in the 2-10µM concentration range.
- The biosensor showed excellent analytical performance with detection and qualification limits of 0.33µM and 2µM, analytical sensitivity of $3.908nA\mu M^{-1}$, repeatability <2.11% and a long-term stability of 14 days.
- The biosensor yielded good results in the determination of uric acid in spiked human serum samples.

3.10. Portable electrochemical biosensor [25]:

- In this work, we developed a highly sensitive electrochemical enzyme electrode that directly determines the uric acid concentration in urine.
- Results from cyclic voltammetry testing showed a well-defined oxidation peak at 0.35 V, but no reduction peak, indicating that an irreversible redox reaction was executed.
- The enzyme electrode's modification method was optimized as follows: 0.1% chitosan, 0.3% graphene oxide, 5 mmol L-1 uricase, and 25% glutaraldehyde.
- Under these optimal conditions, there was a good linear relationship between the redox peak current and uric acid concentration in the range of 0.1-2 mmol L-1 (I=4.1661 CUA + 2.0445; R2=0.9995); and the detection limit was 0.023 mmol L-1 (S/N = 3.3).
- The modified electrode showed high repeatability (RSD = 3.34%), good stability (12 days), and strong anti-interference capability.
- Therefore, this electrode can quickly and accurately determine the uric acid concentration in urine.
- This study is highly significant for monitoring uric acid levels in gout populations and preventing kidney damage caused by gout.

3.11. Grapheme quantum dots as a new substrate for immobilization of uric oxidase [26]:

- A novel strategy for highly sensitive electrochemical detection of uric acid was proposed based on graphene Quantum dot were introduced as a suitable substrate for enzyme immobilization.
- Uric oxidase was immobilized on graphene Quantum dot modified glassy carbon electrode.
- Transmission electron microscope, scanning electron microscopy, cyclic voltammetry and electrochemical impedance spectroscopy techniques were used for characterizing the electrochemical biosensor.
- The developed biosensor responds efficiently to uric acid presence over the concentration linear range 1–800 µM with the detection limit 0.3 µM.

- This novel biosensing platform based on uric oxidase and graphene quantum dots electrode responded even more sensitively than that based on glassy carbon electrode modified by uricase oxidase alone.
- The inexpensive, reliable and sensitive sensing platform based on uric oxidase and graphene quantum dots electrode provides wide potential applications in clinical.

3.12. Nano –au / dna /nano – au / poly (sfr) composite [27]:

- An ultrasensitive method for the simultaneous determination of uric acid was developed using differential pulse voltammetry with a three-dimensionally distributed gold nanoparticle modified glassy carbon electrode.
- The nano-Au/DNA/nano- Au/poly (SFR)/GCE microstructure was characterized by scanning electron microscopy, electrochemical impedance spectroscopy, and atomic force microscopy, showing an anchored three-dimensional distribution of gold nano particles on the modified electrode.
- The electrode exhibited ultrasensitive responses to uric acid due to poly (SFR) electrocatalytic activities and the large surface area of the gold nano particles.
- All four analytes showed well-defined catalytic oxidation peaks at the modified electrode.
- Uric acid yield linear ranges from 9.0×10-9 to1.2×10^{-5}, and detection limits for the analyte was 8.0×10^{-9} M.

3.13. URICASE IMMOBILIZED ON ZnO NANOWIRES [28]:

- In this study, a potentiometric uric acid biosensor was fabricated by immobilization of uricase onto zinc oxide nanowires.
- Zinc oxide nanowires with 80-150 nm in diameter and 900 nm to 1.5 μM in lengths were grown on the surface of a gold coated flexible plastic substrate.
- Uricase was electrostatically immobilized on the surface of well aligned zinc oxide nanowires resulting in a sensitive, selective, stable and reproducible uric acid biosensor.
- The potentiometric response of the zinc oxide sensor *vs.* Ag/AgCl reference electrode was found to be linear over a relatively wide logarithmic concentration range (1 to 650 μM) suitable for human blood serum.
- By applying a Nafion membrane on the sensor the linear range could be extended to 1 to 1000 μM at the expense of an increased response time from 6.25 s to less than 9s, On the other hand the membrane increased the sensor durability considerably.

3.14. Mesoporous Co_3O_4 nanosheets [29]:

- Cobalt oxide nanosheets were synthesized by a facile wet-chemical technique at low temperature in the alkaline phase.
- The cobalt oxidase nanosheets were characterized using various conventional methods, such as Fourier-transform infrared spectroscopy, ultraviolet visible spectroscopy, field emission scanning electron microscopy equipped with X-ray electron dispersive spectroscopy, X-ray photoelectron spectroscopy, Brunauer–Emmett–Teller studies, powder X-ray diffraction, etc.
- Uric acid biological sensors were obtained via the fabrication of a thin layer of nanosheets onto a glassy carbon electrode, surface area: 0.0316 cm^2.
- Improved electrochemical performance such as higher sensitivity, linear dynamic range and long -term stability of the preferred uric acid has been achieved using a reliable I–V method.
- The calibration curves of uric acid are found to be linear (R_2 = 0.901) over a wide range of concentration 0.1 M. Based on a signal-to noise ratio of 3, the sensor sensitivity and limit of detection of uric acid was calculated to be 1.6 × 10^{-4} mA mM^{-1} cm^{-2}, and 60.0 pM.
- Finally, the proposed cobalt oxide nanosheets sensor was applied in the selective detection of uric acid in real sample such as serum and urine and found to give acceptable and reasonable results.

3.15. Amperometry biosensor [30]:

- In this method prepare a biosensor for the determination of uric acid, electro polymerization of pyrrole on Pt surface was carried out with an electrochemical cell containing pyrrole, ferrocene (as an electron mediator) and tetrabutylammonium tetrafluoroborate in acetonitrile by cyclic voltammetry between 0.0 and 1.0V (vs. Ag/AgCl) at a scan rate of 50mV/s upon Pt electrode.
- Uricase was immobilized by a glutaraldehyde/gelatin crosslinking procedure on to polypyrrole film after the electro polymerization processes. The response of the biosensor against uric acid was measured after 330 seconds following the application of a constant potential of +0.7V (vs. Ag/AgCl).
- The resulting biosensor exhibits excellent electrocatalysis for the uric acid.
- The amperometry determination is based on the electrochemical detection of H_2O_2, which is generated in enzymatic reaction of uric acid.
- The sensor responds to uric acid with a detection limit of 5.0×10^{-7} M. The sensor remains relatively stable for 5 weeks.
- Interference effects were investigated on the amperometry response of the biosensor.
- Determination of uric acid was carried out in the biological fluids by biosensor.

III. CONCLUSION:

- In this study, it is concluded that the different methods of electrochemical biosensor used for the estimation of uric acid in human serum, urine and other biological fluids.
- In Point of care testing biosensor is highly used in clinical testing compare to others methods of electrochemical biosensor, because of which gives rapid analysis of uric acid.
- Electrochemical biosensor is directly monitoring the analytes or biological fluids and also monitoring of heavy metals, fluoride, and cyanide and indirectly monitoring organic pesticide or inorganic substances.
- Application of electrochemical biosensor is used in clinical, environmental, industrial and agriculture fields.

- And also have advantages as less of complexity in the sensor setup and economically reliable than others biosensors.
- Because of this electrochemical biosensor useful in estimation of various compounds and biological fluids.

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