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Review Article

Determination of Uric Acid in Biological Fluids with Special Emphasis on Biosensors and Sensors: A Review

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Abstract

Uric acid is produced in human liver from the breakdown of dietary purines and purine compounds into xanthine, which is oxidized by xanthine oxidase to generate uric acid. Since uric acid is the end product of purine breakdown in human, it is discharged through gut and kidneys. The normal uric acid levels in blood are 0.202- 0.416mM in males and 0.142- 0.357mM in females. The increased level of uric acid in serum and urine is viewed as a significant biomarker for gout, hyperuricemia, and renal-disorders, while low uric acid levels affect brain and spinal cord. The determination of unusual uric acid level in blood and urine is required for the differential conclusion and restorative administration of gout, hypertension, cardiovascular ailments, renal infection & Lesch– Nyhan disorder and recently type-2 diabetes. The conventional techniques for uric acid determination are muddled, nonspecific, less touchy and require tedious example pretreatment, costly instrumental set-up and trained people to work, explicitly for chromatographic techniques. The biosensors defeat these downsides, as these are basic, quick, explicit and very sensitive and specific. The uric acid biosensors work ideally within 3–180 s, between5.5–8.5 pH, temperature 22°C - 37°C and uric acid concentration 10-2000mM. These biosensors have been employed to measure uric acid level in serum, urine and saliva, reused up to 200-times within 7–216 days. The future perspectives of uric acid biosensors are discussed.

Keywords: Uric acid; Gout; Uricase; Immobilized uricase; Uric acid biosensors; Uric acid sensors; Serum; Urine; Saliva; Electrochemical methods; Nanomaterials.

INTRODUCTION

Uric acid (UA) [7,9-dihydro1H-purine-2,6,8(3H)- trione] is an end product of purine metabolism, which is re-adsorbed into blood flow framework through essential filtration and incomplete discharge by the kidney. Uric acid in human physiological liquids is of extraordinary significance in determination and treatment of patients experiencing gout and hyperuricemia. It is furthermore a noteworthy cancer prevention agent in individuals. The biochemical oxidation/degradation of uric acid is catalyzed by uricase, orurate oxidase (UOx), found in most warm-blooded creatures. This oxidation changes uric acid to allantoin,

which is exceedingly dissolvable item and discharged through urine. Human lacks uricase, so there is a danger of uric acid accumulation, which leads to hyperuricemia and gout. The ionized type of uric acid in the form of urate is available in blood of which 98% exists as monosodium urate. While low uric acid dimensions might be related with molybdenum insufficiency, copper danger and intensifying of various sclerosis. Along these lines, identification of uric acid dimension broke down in human physiological liquids is crucial for determination of patients experiencing clutters related with modified biosynthesis and catabolism of purine. The microorganisms in the gut can corrupt uric acid, the process called intestinal uricolysis, which accountsto33% of urate transfer. A little urate is found in stools, recommending that uric acid is totally corrupted by intestinal microrganisms. Over 70% of urate is discharged by the kidney. The kidneys' treatment of uric acid is a confused procedure. Urate discharge is improved in women of childbearing age because of the impacts of estrogenic mixes. Men, do nothavea lot bigger uric acid pool than women. The ongoing exploration demonstrates a connection between gout or high blood uric acid dimensions and expanded danger of cardiovascular dreariness and mortality. So, checking of blood uric acid dimensions is of incredible significance. Blood testing is a routine clinical way to deal with deciding blood uric acid dimensions. In excess of 8 million individuals in the U.S., nearly 4% of the populace are assessed to experience the ill effects of gout. The ailment regularly causes extreme torment and physical inability. Gout is related with expanded blood dimensions of uric acid, called hyperuricemia. The ordinary dimension of uric acid in serum is somewhere in the range of 240 and 520mM and 1.4 and 4.4mM in urinary discharge (Raj and Ohsaka, 2003). In male, the typical scope of uric acid in serum is 149~416 µM, which is higher than that of grown-up female's 89~357 µM and kid's 180~300 µM (Grabowska *et al*., 2008). Serum urate ranges in most kids from 3-4 mg/dL (178-238µmol/L). Amid male pubescence, levels start to rise. Female dimensions stay low until menopause. All through adulthood, focuses rise consistently and can shift with stature, circulatory strain, body weight, renal capacity, and liquor consumption. A number of techniques have been reported for measurement of uric acid level in biological materials.

The primary technique for uric acid investigation was colourimetry presented by Offer in 1894.This strategy depends on the synthetic oxidation of uric acid to allantoin, which converts phosphotungstic acid to a tungsten blue chromophore. Nonetheless, this technique experiences a few issues particularly the issue of obstructions, because of different species equipped for creating a similar response (Galba *et al*., 2001). In 1941, Bulger and Johns presented a technique for the assurance of uric acid dependent on the utilization of uricase compound. This strategy depends on the assurance of the decrease, by sans protein filtrates of a basic ferricyanide arrangement, during the oxidation of uric acid by uricase. Among the scientific instruments accessible, a biosensor is the best decision for advantageous measurement of uric acid. Because of the normally low convergence of uric acid that can be removed transdermally, biosensors for this reason ought to in a perfect world have high affectability. A progressively particular methodology is the utilization of uricase, which catalyzes the oxidation of uric acid to allantoin, H_2O_2 and CO_2. The exorbitant creation of uric acid may prompt gout, hyperuricemia and kidney disorder. Different investigative strategies for the detection of uric acid e.g. amperometric, potentiometric, optical, colorimetry, piezoelectric methods, business catalyst terminal and monetarily accessible uric acid unit are utilized generally. A few grids were used by numerous scientists e.g. directing polymers, carbon nanotubes (CNTs), metal nanoparticles (MNPs) electron exchange middle people, bimetallic materials, bienzymes, composite slight films and exhibit setups to upgrade the affectability of biosensors. Wang *et al*. (2020) outlined the importance of uric acid determination and the history of the development of conventional methods foruric acid detection methods such as UV absorption, fluorescence, liquid and gas phase chromatography, capillary electrophoresis, isotope dilution mass spectrometry but limited attention on electrochemical/biosensing methods. Recently, Jamde *et al*. (2025) reviewed different transduction methods such as fluorescence, surface plasma resonance and colourimetric methods for determination of serum uric acid with a focus on how sensitive and portable these techniques are for point of care (POC) analysis. They also explored cost effectiveness, data integration and wireless connectivity for remote monitoring. The present review describes the principle, merits and demerits of all conventional methods of uric acid in biological fluids with special emphasis on biosensing and sensing methods.

CONVENTIONAL TECHNIQUES OF URIC ACID DETERMINATION

The following conventional techniques have been used for uric acid determination such as colourimetry, enzymic colourimetry (Bhargawa *et al*., 1999), UV spectrophotometry (Rocha *et al*., 2010), chemiluminescence (He *et al*., 2005), fluorescence (Galban *et al*., 2001), high performance fluid chromatography (HPLC), HPLC– mass spectrometry (Perelo *et al*., 2005), particle chromatography (Zhao *et al*., 2011), HPLC/isotope weakening mass spectrometry (ID-MS) (Dai *et al*., 2007), hairlike electrophoresis– amperometry (Xu *et al*., 1997), slim electrophoresis with chemiluminescence (Zhao *et al*., 2008). These conventional techniques of uric acid determination in serum and urine are described as follow:

Chemical/Colorimetric Method

The chemical/Benedict method for uric acid determination in blood is based on formation of blue colour in a Folin-Wu blood filtrate, on addition of sodium cyanide solution and special arsenic phosphotungustic acid reagent (Folin, 1930). The method was modified as carbonate-phosphotungustate method. The modified method included a 10min incubation in alkaline solution to destroy non- urate chromogens before addition of phospho-tungustic acid.

Demerit: This technique experiences a few issues particularly the issue of obstructions, because of different species equipped for creating a similar response (Galban *et al*., 2001). The pronounced results are obtainedonly at high concentration of uric acid. The phosphotungstate method was alsoaffected by amino acids e.g. cysteine, tryptophan and tyrosine.

Enzymic Methods Employing Uricase

Enzymatic methods were developed to overcome the problem of chemical methods. All enzymic methods are based on based on following reaction catalyzed by uricase:

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

Enzymic UV Spectrophotometric Method

The uric acid has strong absorption at 293nm. An enzymic spectrophotometric method for uric acid wasbased on decrease in A_{293} by uric acid due to its degradation in presence of uricase (Steel *et al*.1963). Uric acid was determined in serum and urineby this method on a centrifugal analyzer. In this method, the sample was mixed with uricase in a sodium borate buffer,0.05 M, pH 8.5. The resulting decrease in A_{293} was directly proportional to uric acid concentration (Pesce *et al*., 1974).

The other UV (340nm) method of uric acid determination in plasma /serum was based on acetaldehyde reduction by NADH in presence of alcohol dehydrogenase (ADH) The decrease in A_{340}caused by oxidation of NADH was directly proportional to uric acid concentration in serum (Trivedi *et al*., 1978). The following enzymic reactions occurred in this method:

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

$$H_2O_2 + \text{Ethanol} \xrightarrow{\text{Catalase}} \text{Acetaldehyde} + 2H_2O$$

$$\text{Acetaldehyde} + \text{NADH} + H^+ \xrightarrow{\text{Alcohol Dehydrogenase}} \text{Ethanol} + \text{NAD}^+$$

The detection limit of the method was 126mg/L

Demerits: Low precision, low efficiency, Interference by xanthine and background absorbance and high cost.

Enzymic Colourimetric Method

The enzymatic-colorimetric strategy utilizing uricase (UOx) and peroxidase together is broadly utilized for routine due to its effortlessness, affectability and explicitness. In this strategy, uric acid is oxidized aerobically by UOxto generate hydrogen peroxide(H_2O_2), which is measured by a colour reaction consisting of 3-methyl-benzothiazoline-2-one hydrazone (MBTH)/4-aminophenazone/3,5-Dichloro-2hydroxy-benzene sulfonic acid and 3-dimethylaminobenzoic acid (DMAB)/phenol catalyzed by horseradish peroxidase. The colour reaction generates a dye which absorb in visible range500- 520nm. The absorbance of dyeis directly proportional to uric acid concentration (Trivedi *et al*., 1978; Fossati.*et al*., 1980; Huang *et al*., 2004). The following reactions are involved in this method:

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

$$3-\text{Methyl}-\text{benzothiazoline} + 3-\text{dimethylaminobenzoicacid} \xrightarrow{\text{Peroxidase}} \text{Quinoneimine dye (Pink)}$$

OR

$$4-\text{aminophenazone} + \text{phenol} \xrightarrow{\text{Peroxidase}} \text{Quinoneimine dye (Pink)}$$

OR

$$3,5-\text{Dichloro}-2\text{hydroxy}-\text{benzene sulfonic acid} + \text{phenol} \xrightarrow{\text{Peroxidase}} \text{Quinoneimine dye (Pink)}$$

This enzymic colourimetric method has been widely for determination of uric acid in seum and urine employing native enzymes and enzymes immobilized onto free and affixed alkyl/aryl amine glass beads, polyethylene terphthalate membrane, nylon membraneand plasic vial (Trivedi *et al*., 1978; Thomasson *et al*., 1982; Bhargava *et al*., 1999; Pundir and Bhargava, 2003; Sushma *et al*., 2004; Phaugat *et al*., 2010; Bhargava and Pundir, 2012; Chauhan *et al*., 2014). The detection limit of the method was 1.2 mmol/L

Demerits: The test packs of this strategy are financially accessible, the expense of uricase and peroxidase utilized in the packs is a factor that limits across the board utilization of the technique for large number of tests. Further, the method is time consuming and had interference by certain endogenous substances found in biological fluids.

Enzymic Spectrofluorometric Method

An enzymic spectrofluorometric method for determination of uric acid in plasma was reported by Kamoun *et al*., 1976.In this method, uric acid is oxidized by uricase to form H_2O_2, which get condenses by oxidation of two molecules

of p-hydoxypenil acetic acid in the presence of peroxidase. The product formed (Dicarboxymethyl-5,5'dihydroxy2,2'biphenyl) is fluorescent and has excitation wavelength at 330nm and emission wavelength at 430nm.The fluorescent intensity is measured in a spectrofluorometer, which is directly proportional to uric acid concentration. It is ultramicro method and can measure uric acid in plasma upto50 ng in 10ul sample. The Following reactions are involved in this method:

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

$$2H_2O + 2\,p-Hydoxyphenilacetic\ acid \xrightarrow{\text{Peroxidase}} + \text{Dicarboxymethyl} - 5,5'\text{dihyroxy2,2'biphenyl}\ 4H_2O_2\ \text{Fluorescent}\ (Ex = 330nm, Em = 430nm)$$

Odo *et al*., 2004 employed this method for determination of uric acid in serum, usingFe^{3+} -thiacalixarene tetrasulfonate complex in place of peroxidase. The method showed a linearity for uric acid in the concentration range, 0.5 to 5.0 µgm/ml.

Chemiluminescence

Luminescence is a result of chemical change, when light emits with restricted emission of warmth. Chemiluminiscence is an outstanding and prominent explanatory technique as a result of its high affectability, low location limit, wide direct working reach, and its rate, just as the way that it very well may be performed with generally basic and reasonable instrumentation, as an excitation source and optical channels are not required. It has been generally connected in different fields, including clinical conclusion, biotechnology, pharmacology, sanitation, and ecological science. A novel chemiluminescence (CL) sensor based on flow injection analysis was developed for measurement of uric acid level in human urine and serum using controlled-reagent-release technology. The reagents involved in the chemiluminescence (CL) reaction, luminol and periodate, were immobilized onto anion-exchange resin column. The chemiluminescence generated by released luminol and periodate upon injection of water in alkaline media was inhibited in presence of uric acid. The decreased chemiluminescence (CL) intensity was directly proportional to uric acid concentration with linearity in the uric acid concentration range, 5.0-500.0 ng/ mL and a detection limit of 1.8 ng/ mL). The flow sensor could be easily reused for over 80 h with sampling frequency of 100/ hr. The sensor was employed for determination of uric acid in human urine and serum with RSD < 3.0% (Song and Hou, 2002).

A chemiluminescence method was developed for determination of uric acid, based on its inhibitory effect on the catalysis of CO_2+ for the oxidation of luminol by H_2O_2. The method was simple, highly sensitive and cheaper. The decrease of chemiluminescence intensity was directly related to uric acid concentration. The linearity was in the range of 1.0 x 10^{-10} - 7.0 x 10^{-6} M and the detection limit was1.1 x 10^{-11} M. The relative standard deviation (RSD) for 5.0 x 10^{-8} was 1.9% (n=4). The method was applied for measurement of uric acid level in human urine and serum (Guang *et al*., 2005).

HPLC

High performance liquid chromatography (HPLC) is a highly improved form of column chromatography. Instead of solvent being allowed to drip through a column under gravity, it is forced through under high pressure up to 400 atmospheres. It allows using a very much smaller particle size for the column packing material for column, which gives a much greater surface area for interaction between stationary phase and flowing molecules through it. In HPLC, the mobile phase is a solvent. This solvent is pumped through a column and the stationary phase is a finely divided solid held inside the column. A precise method for determination on uric acid in serum was developed based on direct electrochemical oxidation in the elute from HPLC. (Pachla *et al*., 1975). Uric acid in human saliva was determined by reversed-phase HPLC with amperometric electrochemical detection (Inoue *et al*., 2003).

A simple and direct determination of uric acid method in various biological matrices based on HPLC-mass spectrometry was reported. Chromatographic separations were performed with a stationary phase Zorbax Sax column (an ion exchange resin) with 50% sodium citrate 1mM, pH6.5 and 50%acetonitrile as mobile phase (Perello *et al*., 2005).

A simple HPLC method was developed and validated for determination of uric acid in human serum (Cooper *et al*., 2006). An improved HPLC method for simultaneous quantification of allantoin, uric acid and creatinine in cattle urine was developed (George *et al*., 2006). Uric acid level was also measured in serum using HPLC coupled with isotope dilution mass spectrometry, as a candidate reference method (Dai *et al*., 2007).

Demerits: Requirements of **e**xpensive equipment and trained person to operate, time consuming sample preparation.

Oscillating Reaction Method

It is a new analytical method for determination of uric acid by the perturbation of uric acid on Belousov-Zhabotingsky oscillating reaction. The method is based on the linear relationship between the changes in the oscillating period and uric acid concentration. The calibration curve was linear over the range of 20x10^{-5} to 5.0x10^{-4}M. The method showed a detection limit of 3.28x10^{-6}M and good precision

(RSD=3.6%) and excellent throughput (10 samples / hour) (Wang *et al*.,2005)

Demerits: Malonic acid, potassium bromide, sulfuric acid and ferroin solution used in oscillation reaction are very harmful.

Capillary/Slender Electrophoresis

Capillary/slender electrophoresis (CE) encompasses a family of related separation techniques that has use narrow-bore fused silica capillaries to separate a complex array of large and small molecules. High electric field strengths are used to separate molecules based on differences in charge, size and hydrophobicity. Sample is introduced by immersing the end of capillary into a sample vial and applying pressure, vacuum or voltage. Depending on the types of capillary and electrolytes used, the CE can be segmented into several separation techniques. A fast free zone CE method for simultaneous determination of uric acid and ascorbic acid in human plasma was reported. This method was compared with a validated CE by measuring uric acid and ascorbic acid in plasma of 32 normal persons and the data obtained was analyzed by Passing and Bablok regression (Zinellu, *et al*., 2004).

A simple and sensitive method based on CE with chemiluminiscence (CL) detection was developed for determination of uric acid. The sensitive detection of uric acid was based on the enhancement effect of uric acid on CL reaction between luminal and potassium ferricyanide in alkaline solution. The method was evaluated by quantifying uric acid in serum and urine with satisfactory assay results. The lower detection limit of the method was $5x10^{-6}$M (Zhao *et al*., 2008)

Demerits: Expensive chemicals and expertise handling.

Isotope Dilution Mass Spectrometric Method

In isotope dilution mass spectrometric method for uric acid, a known amount of ^{15}N labeled uric acid is added to serum with unknown amount of uric acid (in sample to be tested). The mixture is allowed to equilibrate. Isotopes ratio measurements are made from the abundance of the [M-tert-butryl]$^{+}$ ions at m/z567 and 569.Bias characteristics are analyzed by measuring uric acid level in the samples under different chromatographic conditions and with different ionization techniques. Uric acid was determined in 3 lyophilized sera by this method. For standard reference material (SRM)909, 4 sets of 6 samples were prepared. The coefficient of variation (CV) for a single measurement ranged from).34 -0.42%, while the relative standard error of the mean ranged from 0.08 to 0.14% (Ellerbe *et al*., 1990).

Demerits: Radioactive material are expensive and requires expertise handling, require expensive instruments, cumbersome and time consuming.

Radioisotope Mass Fragmanography

In this method, urine samples contain ^{15}N labeled uric acid and its oxidation product, allantoin. The uric acid and allantoin are isolated using an AG1-X8(C1-form) anion exchange column and heated with a mixture containing 1:1 dimethylformide and N-(tert-butyldimethylsilyl)-N-trifluroacetamide (MTBSTFA). The ter-butyl dimethylsilyl (TBDMS) derivative of allantoin and uric acid are formed, which are injected into a gas-chromatograph interfaced with a mass spectrometer, operated under electron impact ionization conditions. The quantification of allantoin and uric acid is based on isotopic dilution by spiking the urine sample with known quantities of 99 at % ^{15}N-labelled uric acid and allantoin internal standards. A method for determination of ^{15}N enrichment and concentration of allantoin and uric acid simultaneously in urine using gas chromatography/mass spectrometry (GCMS) is reported. CV in measurements of isotope ratio and concentration were 0.2% and 0.5% respectively. The method was also employed to measure urinary recovery of [1,3-^{15}N] uric acid (Chen *et al*., 1998)

Demerits: Expertise handling of radioactive material, use of costly chemicals and and equipments and instruments, cumbersome and time consuming.

All these conventional techniques are generally difficult, costly, tedious, time consuming, sometime nonspecific and require expensive equipment and trained persons to operate. Biosensors overcome these problems

BIOSENSORS AND SENSORS FOR URIC ACID QUANTIFICATION

Biosensors consist of biological entity, which could be an enzyme, antibody, protein, nucleic acid or a cell organelle, cell (from animal or plant), tissue slices, and microorganism that interacts with an analyte and produces a signal, which can bemeasured. The biosensor has two elements:1) Bioelement and 2) Sensor element. The bioelement is a biological component, which acts as a sensor, transduces and detects the signal. The biosensors can be classified on the basis of transducers such as electrochemical, calorimetric, optical, and piezoelectric dependent on the transducer utilized (Mehrvar *et al*., 2004; Buerk *et al*., 1993; Conroy, 2009). In electrochemical biosensors, the flag delivered by the collaboration of an organic component and substrate can be estimated by an electrochemical indicator (Arya *et al*., 2012). Calorimetric biosensors depend on the estimation of warmth going with a biochemical response (Lai *et al*., 2011). Optical biosensors depend on the estimation of adsorbed or produced light coming about because of a biochemical response (Zhao *et al*.,2017) Piezoelectric biosensors are mass touchy and identify the adjustment in recurrence of wavering after adsorption or desorption of analyte particles on the outside of piezoelectric finder (Lu *et al*., 2011). Recently uric acid

biosensors utilizing nanomaterials and nanostructures have showed their high compelling floor territory, improvement of mass shipping and notable conductivity (Falasa, 2006; Merrimen and Dalbeth, 2011; Nakagawa *et al*., 2006; Nyhan, 1997; Jossa *et al*., 1994; Lohsoonthorn *et al*., 2006; Gagliardi *et al*., 2009). The gold nanoparticles (AuNPs) showed first rate electron trade efficiency among protein and terminal floor, which activates extensive applications in electrochemical biosensors (Fang and Alderman, 2000). In uric acid biosensors, uricase (UOx) from numerous sources, such as *Arthrobacter globiformis* (Kanyong *et al*., 2012), *Bacillus fastidiosus* (Luo *et al*., 2006), *Aspergillus niger* (Liang *et al*., 2007) and *Candida sp.* (Chen *et al*., 2008) and porcine liver has been applied as biosensing element, which catalyze the following reaction:

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

A number of biosensor systems aimed at the specific measurement of uric acid are discussed below:

Dissolved Oxygen (DO) (O_2) Consumption Based Biosensors

The first amperometric technique for quantitative analysis of uric acid in organic liquids was reported by Nanjo and Guilbault in 1974. O_2 consumption was measured on a Clark type O_2 anode comprising of a Pt cathode, where O_2 is diminished, and a reference terminal, normally Ag/AgCl is used. At the point, when a capability of - 0.60 V versus Ag/AgCl is connected to the Pt terminal, O_2 is diminished by response and present corresponding to O_2 fixation is created. The outcomes for uric acid in urine by utilizing the biosensor was focused. The interfering substances like glucose, urea, ascorbic acid, lactic acid, glycine, KCl, NaCl, $CaCl_2$, $MgSO_4$, and NH_4Cl demonstrated no obstructions on the reaction of the uric acid biosensor. The epoxy resin membrane was accounted for immobilization of uricase, because of its high liking for catalyst, high temperature stability, porosity, ease, and concoction opposition. In O_2 assurance-based investigations, impedance impact isn't extremely normal. The reaction of the anode relies upon the O_2 focus in the arrangement and this can diminish the precision of the biosensor. The most normally utilized choice to conquer this downside is the recognition of H_2O_2.

DO metric uric acid biosensor rebased on immobilized uricase onto membrane, sensing part of combined electrode of DO meter.

Principle: The membrane-type dissolved O_2 electrodes using a galvanic cell was configured as illustrated below. The working electrode used was a noble metal (Ag), and the opposite electrode used a base metal (Pt). Forth electrolyte, an alkaline solution was used and forth membrane, a highly oxygen-permeable Teflon membrane was used. O_2 which has passed through the membrane was reduced with the working electrode. A reduction current in proportion to the concentration of the dissolved O_2 was generated and then the dissolved O_2 was measured (Fig.1).

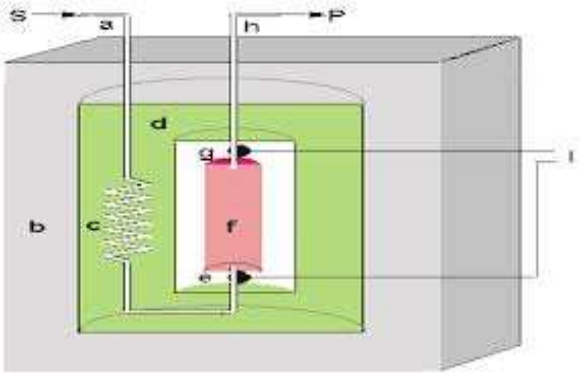

Fig. 1: Schematic representation of dissolved O2 (DO) meter-based biosensor

Membrane Based DO Meteric Electrode

Clark Type Oxygen Sensing Teflon Membrane Electrode

The uricase was immobilized through crosslinking on the working electrode. The biosensor was applied for the uric acid determination in serum. (Nanjo and Guilbault, 1974). A biosensor for the specific determination of uric acid in urine was developed using uricase/urate oxidase (UOx)(EC1.7.3.3) in combination with a dissolved O_2 probe. UOx oxidase was immobilized with gelatin by means of glutaraldehyde and fixed on a pretreated Teflon membrane to serve as enzyme electrode. The biosensor had following characteristics: ***Optimum pH:*** pH 9.2 in 0.05M glycine buffer, ***Optimum temperature*** 35ºC, ***Linearity:*** 5-40 µM, ***Response time:*** 5 min, ***Stability:*** > 2 weeks during which 35 assays were performed (Dinçkaya *et al.,*2000)**.**

2.1.2 Egg membrane bound oxygen electrode

Uricase from *Arthrobactor globiformi* was immobilized onto eggshell membrane by glutaradehyde crosslinking. A uric acid biosensor was fabricated from egg membrane bound uricase by binding onto oxygen electrode. The detection scheme included the enzymatic reaction of the uricase leading to the depletion of dissolved O_2 level upon exposure to uric acid solution. The decrease in O_2 level was monitored, which was directly related to the uric acid concentration. The biosensor had following characteristics: Optimum pH: 8.0 Optimum temp: 30ºC, Linearity**:** 4.0–640µM, Minimum Detection limit (LOD): 2.0 µM (S/N=3), Response time 100s. Interference: No interference by common potential interferents in samples suc has glucose, urea, ascorbic acid, lactic acid, glycine, D, L-alanine, D, L-cysteine, KCl, NaCl, $CaCl_2$, $MgSO_4$, and NH_4Cl. The biosensor was employed for uric acid determination in serum and urine samples. The biosensor showed extremely good stability with a shelf-life of at least 3 months (Zhang *et al.,* 2007).

Silk Fibroin Membrane on Oxygen Electrode

An amperometric urate biosensor was described based on porcine liver uricase immobilized silk fibroin membrane

mounted over an O_2 electrode inf low injection analysis. Type of immobilization wasliquid entrapment. The biosensor had following characteristics: *Optimum* pH 9.0, *Optimum Temp* 32ºC, *Linearity*from 0.2mmol/L to1.0mmol/L, limit of detection (LOD) was 0.2mmol/L, *Interference s*lightly by ascorbic acid, *Application* The biosensor was used for determination of serum and urine uric acid. *Recovery* 94.2-102.6%and92.2-97.9%recoveriesof added uric acid in serum and urine respectively. The sensor was capable of detecting 60-70 human serum samples/hr *Stability* 3-4 months (Zhang *et al.,* 1998).

Epoxy Resin Membrane on Combined Oxygen Electrode

Cow pea leaf uricase was immobilized on to epoxy resin membrane with 80% retention of its initial activity. The uricase epoxy resin bioconjugate membrane was mounted over the sensing part of the combined electrode of dissolved O_2 meter to develop a uric acid biosensor. The biosensor exhibited optimum response within 10-12s at a pH 8.5 and 35ºC. There was a linearity between uric acid concentration from 0.025 to 0.1 mM and O_2 (mg/l) consumed. The biosensor measured of uric acid in sera, which was. 4.92 mg/dl in apparently healthy males and 3.11 mg/dl in females. The analytic recoveries of added uric acid in reaction mixture (8.9 and 9.8 mg/dl) were 93.6 % and 87.18 % respectively. The within and between batch coefficient of variation (CVs) were < 6.5 and < 5.0%, respectively. The biosensor showed a good correlation (R^2 =0.996) for serum uric acid value with standard enzymic colorimetric method. Among the various metabolites tested only, glucose (11%), urea (38%), NaCl (25%) and cholesterol (13%) and ascorbic acid (56%) caused decrease, while, $MgSO_4$ and $CaCl_2$ had no effect. The enzyme electrode exhibited only 32% decrease in its initial activity, after its 100 uses over a period of 60 days, when stored at 4ºC (Arora *et al*., 2009).

PVC Membrane on Combined Oxygen Electrode

A DO metric uric acid biosensor was developed by immobilizing uricase onto PVC membrane and then mounting this conjugate over sensing part of combined electrode of O_2 meter. The biosensor showed optimum response within 10-12s at pH 9.0 and 35ºC.There was a linearity between O_2 (mg/l) consumed and uric acid concentrations in the range, 0.1 to 0.8mg/dl (Lower range) and 1.0-10.0 mg/l (Higher range). The biosensor measured uric acid level in sera of apparently healthy male (6.1mg/dl) and female (4.9 mg/dl). The enzyme electrode lost 32% of its initial activity, after its 200 uses, when stored at 4ºC (Bhawna and Pundir, 2010)

Amperometric Biosensor

Among all the biosensors available, amperometric sensors, which monitor the change in current at fixed voltage induced by redox reaction, are most popular (Cunningham, 1999). The popularity of amperometric sensors can be explained largely on the basis of their simplicity, ease of production and the low cost. The signal in amperometric devices depends on the rate of mass transfer to the electrode surface. In order to minimize the diffusion path of the detectable product of the reaction, the enzyme requires being in close contact with the transducer (Bardeletti *et al.,* 1991).

Principle:

H_2O_2 generated by oxidation of uric acid by uricase, provides current under high potential, which is directly proportional to uric acid conc.

$$\text{Uric acid} + H_2O \xrightarrow{\text{Uricase}} \text{Allantoin} + H_2O_2$$

$$H_2O_2 \xrightarrow{\text{High potential}} O_2 + 2H^+ + 2e\text{-(Current)}$$

Amperometric Uric Acid Biosensor Based on Transducer

Various amperometric uric acid biosensors have been reported for the rapid determination of uric acid based on current measurement produced by electyrocatalytic reduction of H_2O_2. Such uric acid biosensors with different transducers are discussed below:

Thin Film of Polyaniline and Polypyrrole on Carbon Felt:

In such transducer aniline and pyrrole solution containing uricase at a neutral pH was made to immobilize onto the surface of gas diffusion carbon felt. The selective uric acid sensor was fabricated by carbon felt bound uricase and an O_2 electrode with O_2 permeable membrane. The biosensor had following characteristics: ***Response Time:*** <5min, ***Linearity***: Concentration range of 1×10^{-5} to 1×10^{-4} M, LOD: 4×10^{-6} M (Uchiyama *et al.,* 1997)

PVA-SbQ Membrane:

In this electrochemical biosensor, uricase was entrapped in poly (vinyl alcohol) N-methyl-4(4'-formylstyryl) pyridinium methosulfateacetal (PVA-SbQ), a cationic polymer matrix to detect uric acid for wound monitoring. The polymer-enzyme ratio for immobilization was 53.2 µg cm^{-2}: 0.25 U cm^{-2}. Uric acid was detected both optically as well as electrochemically. A redox electron shuttle, ferrocene carboxylic acid (FCA) was used to facilitate electron transfer. Entrapped uricase provided improved response to uric acid detection compared to physisorbed uricase. There was a linearity between biosensor response and uric acid concentration between 12 and 100 µM. The biosensor was stable for 48 h and maintained 90% activity until 5 days. The biosensor measured uric acid in biofluids of sweat and wounds. The analytical recovery of added uric acid in these bio-fluids was ~102–107 %. (RoyChoudhury *et al.*.,2018).

Self-Assembled Monolayer on Au Electrode:

In such transducer, uricase molcecules onto self-assembled monolayer of 2- aminoethanethiolate were prepared on an Au electrode by casting phosphate buffer solution containing uricase and glutaraldehyde as a cross-linking agent, followed by drying. The prepared electrode

(uricase/Au) had amperometric responses to uric acid with use of $[Fe(CN)6]^{3-}$ dissolved in solution as an electron mediator at rates much higher than those obtained with use of dissolved uricase. The sensitivity of the uricase/Au electrode was influenced by the amount of glutaraldehyde in the casting solution but also by the drying time and the temperature after casting of the solution. The biosensor had following characteristics: Km^{app}: 0.90 mmol dm^{-3}, ***I max:*** 85.0µ Acm^{-2} ***Linearity:***0.1 mM dm^{-3} to 0.6 mM dm^{-3} ***LOD:*** 0.6 mM dm^{-3}, which covers the normal concentration of uric acid in the human blood **(**Kwuabata *et al.,* 1998)

Polyaniline (PANI) Layer-Based Electrode:

Polyaniline (PANI) based uric acid biosensor was prepared by template process. Firstly, a PANI–uricase electrode was obtained using one-step process. Secondly, the electrode was hydrolyzed in 6.0 mol/dm^3 HCl solution to remove the uricase that may be affected by aniline monomer from PANI film. Finally, Commercial uricase from *Aspergillus Niger*, was immobilized into the PANI film, based on the principle of the doping and undoping of the conducting polymer and PANI–uricase biosensor was obtained. The biosensor had following characteristics: ***Optimum pH:*** 9.6, ***Response time*** <10sec, ***Km:***2.31 mol/dm3, ***Imax***: 3.49µA***Linearity:*** 0.0036 to 1.0 mmol/dm^3, ***Stability:*** 18% for 60 days, in case of one step and 39% for 40 h in case of two-step process (Kan *et al.,* 2004).

A selective uricase biosensor was developed by entaping A.niger uricase onto electroactive intrinsic PANI film at high pH. The well-known interferents showed no measurable effect on the biosensor's current response. In the range from 0.20 to 0.50V, the current response increased with increase in potential. In the wide pH range from 2 to 12, current response increased with increasing pH, and the optimum pH was not appearing and response of the biosensor was checked at pH 7.0. The biosensor showed following analytic characteristics: ***Optimum pH***: 7.0, ***Km:*** 7.83 mmol dm^{-3} ***Imax:*** 58.07 µA, ***Linearity:*** 1.0×10^{-3} to 10×10^{-3} dm^{-3}. ***Interference:*** No inference from ascorbate, ***Stability:*** Stable for 157 days (Jiang *et al.*, 2007)

1) PANI/PB Modified Pt Electrode

A uric acid biosensor was developed by electrodepositing polyaniline/prussian blue (PANI-PB) composite on a Pt electrode and then immobilizing uricase onto this composite. The working of the electrode was tested by cyclic voltammetry (CV) and found to be more effective than the PANI-modified electrode in the low-potential range. The biosensor gave a linear response for uric acid in the concentration range 10–160 µM with a sensitivity of 160 µA mM^{-1} cm^{-2}. The major advantage of this PANI-PB composite electrode was is its low working potential (0 V vs. Ag/AgCl) for determination of uric acid and insensitivity towards possible interfering analytes found in real samples. The biosensor was selective to uric acid and had no interference by ascorbic acid, glucose, and urea. The sensor electrode retained its selectivity and sensitivity for 7days, when stored at −18 °C. The biosensor was employed for determination of uric acid in human sera (Thakur and Sawant, 2013).

2) Polyaniline-Poly (n Butyl Methacrylate) Bio Composites

Polyaniline-poly (n butyl methacrylate) (PAN-pBMA) composites films were obtained by casting. The electric conductivity and mechanical properties of film were studied. Porcine liver uricase was immobilized onto this composite film by adsorption. The composite film was applied as uric acid biosensor. The biosensor exhibited following characteristics: ***Linearity:*** 5 to 12.5 mg/dl, (Castillo *et al.,* 2002).

3) PANI Films on ITO Coated Glass Plate

Commercial uricase from *B. fastidius* was immobilized using glutaraldehyde as cross-linker, onto electrochemically synthesized PANI films to construct working electrode. The morphology and covalent linkage of uricase lead to high enzyme loading and better shelf life. Km obtained was 5.1×10^{-3} mM L^{-1} for immobilized uricase compared to that of free uricase (3.4×10^{-1} mM L^{-1}), which suggested enhancement in affinity and/or activity of uricase attached to PANI. The biosensor had following characteristics: Optimum ***pH:*** 6.5, Optimum ***Temp:*** 35 ºC, Response ***time:*** 60s, ***Linearity:*** 0.01-0.05mM, LOD= 0.01mM, the biosensor measured uric acid in blood serum ***Stability& reusability:*** 90% retention of activity after 30 uses during 17–18 weeks, when stored at 4 ºC (Arora *et al.,* 2007).

4) Polyaniline-Polypyrrole (PANI-PPy) Film on Pt Electrode

A new amperometric uric acid biosensor was developed by immobilizing uricase through glutaraldehyde crosslinking procedure on PANI-PPy composite film on the surface of a Pt electrode. Uric acid was determined by the oxidation of enzymatically generated H_2O_2 at 0.4 V vs. Ag/AgCl. The biosensor showed following characteristics: Optimum pH: 7.5, ***Response time:*** 70s, ***Linearity:*** 2.5×10^{-6} – 8.5×10^{-5} M LOD: 2.5×10^{-6}M, ***Interference:*** 31% interference by paracetamol. ***Stability:*** 80% at the end of the 4th week. (Arslan, 2008)

Metal Film Based Electrode:

1) ZnO Thin Film and CuO Microclusters Arrayed p-n Heterojunction

An array of p–n junction heterostructures was synthesized by loading the surface of n-type semiconducting ZnO thin

film with p-type CuO microclusters (μCs) without any external mediator. Uricase was immobilized onto this prepared CuO/ZnO arrayed heterostructure for fabrication of a uric acid biosenor. The electrochemical performance of the prepared *p-n* junction-based biosensor was carried out by CV. A stable and well-defined peak corresponding to oxidation of uric acid was observed at a very low potential of 0.03 V in a mediator free solution. The decrease in working potential of biosensor was due to the substantial enhancement of surface reaction kinetics in the formed *p-n* junction heterostructure due to built-in electric field. This facilitated the charge transport. There was linearity in the uric acid range of 0.05–1.0 mM, high sensitivity of 1.74 mA/mM. The biosensor had a good affinity towards uric acid ($Km\sim$ 0.05 mM) and possesses long shelf life (>20 weeks). The low operating potential (0.03 V) of CuO/ZnO arrayed *p-n* heterojunction decreased the energy consumption of developed biosensor and efficiently minimized the interference from common oxidizable interferents, and hence making the biosensor highly selective (Jindal *et al*., 2017).

2) *Au Coated Cu Electrode*

A new second generation uric acid biosensor was developed for detection of urinary uric by chemically binding both uricase and redox mediator to inexpensive copper wire through simple electrodeposition of gold on copper surface and subsequent functionalization of the gold with L-methionine. The biosensor showed an average of a low oxidation potential of 0.33 V, a response time of 5 s, a widest linearity for uric acid in the concentration range 0–2.38 mM, with a LOD of 2.4 μM and a sensitivity of 50 μA mM^{-1}, and The within and between batch assay of uric acid determination of uric acid in human urine by this biosensor were 85.6–95.5 % and 0.3–2.4 %, respectively and correlation with a standard method was R^2=0.9952.The biosensor was used successfully for period of 209-day (Cheng and Kao ,2016).

3) *Screen-Printed Carbon Electrode (SPCE)*

A methodology was devised for simultaneous determinations of purine bases of hypoxanthine, xanthine, and uric acid with preanodizednontronite-52 coated screen-printed carbon electrodes (NSPEs). A good enhancement in the electrochemical sensitivity was found upon preanodization at 2.0V versus Ag/AgCl. Cyclic voltammetric studies showed different charge transfer behavior among the purine bases on the NSPE. The experimental parameters were systematically optimized for each analyte, hypoxanthine, xanthine, and uric acid. Commercial uricase from *Arthrobactor globiformis* was immobilized onto SPCE by glutaraldehyde crosslinking. The biosensor had following characteristics: ***Linearity:*** 2-40 mM, LOD: 0.42 mM. Analytic ***Recovery:*** 95.4 %, 98.25% and 101.3% respectively in denatured plasma, plasma and urine the biosensor measured uric acid in denatured plasma, plasma and urine (Zen *et al.,* 2002)

4) *Screen printed Electrode (SPE)*

A non-enzymatic detecting electrode strip was reported for fast monitoring of uric acid in human whole blood. A single-use amperometric uric acid sensor strip, incorporating a three-electrode configuration was fabricated on a polypropylene substrate using low-cost screen printing (thick-film) technology. Both the working and counter electrodes were prepared by screen-printing commercial carbon ink.The biosensor had following characteristics: ***Response Time:*** 5s, ***Interference:*** No interference by ascorbic acid (Chen *et. al.,* 2005)

Han *et al.*.(2022 introduced a uricase immobilized paper (UOx-Paper) integrated electrochemical sensor for detection of uric acid. The UOx-paper was integrated with a Pursian Blue (PB) modified SPCE by electro-polymerization of phenylenediamine .The biosensor had sensitivity of 4.9 μM/mM and linearity of 50-1000μM with a LOD of 18.7μM,It was applied for detection of uric acid in saliva of apparently healthy and gout patients, The results were verified by conventional methods electrode

5) *Electropolymerized Pyrrole on Pt Electrode*

The electropolymerization of pyrrole on Pt surface with an electrochemical cell containing pyrrole, ferrocene (as electron mediator) and tetrabutylammonium tetrafluoroborat in acetonitrile was carried out. The electrode was characterized by cyclic voltammetry between 0.0 and 1.0V (vs. Ag/AgCl) at a scan rate of 50mV/s upon Pt electrode. Commercial bacterial uricase was immobilized by a glutaraldehyde-gelatin crosslinking on to polypyrrole (PPy) film after the electropolymerization 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 exhibited excellent electrocatalysis for the uric acid. The amperometric determination was based on the electrochemical detection of H_2O_2, which is generated in enzymatic oxidation of uric acid. The biosensor had following characteristics: ***Optimum pH***: 8.0, Response time: 330s, Optimum temp: 50ºC, K_m: 0.44mM, V_{max}:7.1 $\times 10^{-2}$ mM/min, LOD: 5.0 $\times 10^{-7}$ M**,** ***Interference:*** No interference from ascorbic acid, ***Stability*** 5 weeks (Cete *et al.,* 2006).

6) *Ir-Modified Carbon Electrode*

An amperometric biosensor based on an Ir-modified carbon (Ir–C) working electrode with immobilizing *Bascillus fastidious* uricase by thick film screen printing technique (Entrapment) was developed. The chronoamperometric method was used to make uricase/Ir-C electrode for determination of uric acid. The high selectivity of uric acid biosensor was achieved due to the reduction of H_2O_2 oxidation potential based on Ir-C electrode. Using uricase/Ir-

C as the sensing electrode, the interference from ascorbic acid was slight at the sensing potential of 0.25V (versus Ag/AgCl). Uric acid was detected amperometrically based on uricase/ Ir–C electrode with a sensitivity of 16.60 AmM^{-1}. The biosensor had following characteristics: ***Optimum pH***: 7.0, ***Optimum Temp:*** 37ºC, ***Response time:*** 24.7-40.8s, ***Interference:*** Insignificant interference ascorbic acid, Linearity***:*** 0.1-0.8 mM, LOD:0.01mM (S/N = 6.18) ***Stability:*** Up to 10 days (Luo *et al.,* 2006).

Poly-O-Aminophenol-Aniline Copolymer:

A poly-*o*-aminophenol-aniline based uricase biosensor was proposed. An uricase electrode was prepared with one-step process using the copolymer of aniline and *o*- aminophenol, electrode was washed in 6.0 mol dm^{-3} HCl solution to remove the uricase that may be affected by monomer during copolymerization and a template with moderate apertures for immobilization of uricase. Finally, active uricase (from porcine liver) was immobilized by electrochemical entrapment onto the template based on doping and undoping of the copolymer, to fabricate a copolymer-uricase biosensor. The biosensor had following characteristics: ***Optimum pH:*** 65-8.5, ***Km*** 10.08mmol dm-3 ***Imax***: 26.53µA**,** ***Linearity***: 0.001 to 0.1 mmoldm^{-3} ***Stability:*** For the copolymer-uricase biosensor prepared with template process was 19% for 50 days (Pan *et al.,* 2006).

Indium Tin Oxide Substrates Coated with a Layer of Prussian Blue:

A layer-by-layer technique for immobilizing the B.subtilis uricase immobilized onto ITO substrates coated with a layer of prussian blue (PB) by entrapment. Uricase layers were alternated with either poly (ethylene amine) or poly (di-allyl, di-methyl ammonium chloride), and the resulting films were used as amperometric biosensors for uric acid. The biosensor had following characteristics: ***Response time:*** 2-6s, ***Linearity:*** 0.1 and 0.6 M of uric acid, LOD: 0.15 µ mol L^{-1} cm^{-2}, ***Interference:*** Negligible influence from interferents, as detection was carried out at 0.0 V vs saturated calomel electrode. ***Stability:*** Stable for 10 days (Marli *et al.,* 2007).

Gold Electrode Coated with Polystyrene:

A novel amperometric uric acid sensor was prepared by coating the surface of an Au electrode with a polystyrene membrane (PS). The PS membrane formed using 30 mg mL^{-1} PS chloroform solution combined with 30 µl of poly maleimido styrene (PMS) solution (5mg ml^{-1}) as a dispersant for uricase. This membrane was successfully employed as an immobilization support for uricase. In this membrane, PMS formed micelle-like structures containing uricase in an active state. This immobilized uricase membrane permitted the permeation of O_2, consumed by the uricase reaction. The biosensor had following characteristics: ***Optimum pH***: 7.0, ***Linearity:*** 5-105µ M, ***Interference:*** No ascorbic acid interference, ***Stability*****:** Stable for 21 days (Wang *et al.,* 2007).

Zang *et al.* (2021) developed highly sensitive uric acid biosensor based on grapheme chemoregister and magnetic beads (MB). Uricase was immobilized onto MB (single layer graphene and HfO_2 thin film, could decouple the functionalization steps from sensor surface, which allow sensor to be reusable. This sensor detected the change in ph. The biosensor showed a linearity in the range 1-1000µM with a LOD of 1.0µM and high sensitivity of 5.6 mV/decade and excellent linearity with standard method (R^2=0.9945).The biosensor was unaffected by glucose and urea.

Amperometric Biosensor for Salivary Uric Acid:

A uric acid biosensor aimed at a simplified determination of salivary uric acid was fabricated and utilized for the measurement of the diurnal variation of salivary uric acid. The biosensor measures uric acid as the change in the amount of hydrogen peroxide produced in the uricase reaction. Because uric acid is oxidized as easily as hydrogen peroxide, the osmium-HRP redox reaction was employed. The sensitivity of the biosensor was 170 nA/mM, which was sufficient for salivary uric acid determination. For simplified measurement of a saliva sample, a paper based saliva sampling device, which enables the sample collection of a regulated amount of saliva in 5 s, was used. As a test using an actual sample, the diurnal variation of salivary uric acid was measured. The result indicated that salivary lactic acid increased in the morning. The total measurement time for the saliva measurement was approximately 3 min, which was sufficiently fast for the purpose of daily health management. The method was used not only in gout treatment but also possibly in the measurement of other substances contained in saliva (Kudo and Takagi, 2018).

Nano-Materials Based Biosensors:

1) Inorganic Nanomaterials based biosensors

A. ZnO Nanorodes

A reagent less uric acid biosensor was prepared based on *Arthrobactor globiformis* uricase immobilized onto ZnO nanorodes by crosslinking. The biosensor had following characteristics: ***Optimum pH:*** 6.5, ***Linearity*** 5.0×10^{-6} to 1.0×10^{-3} mol/L, LOD: 2.0×10^{-6} mol/L. ***Interference*** No interference by ascorbic acid as it was successfully separated by uricase/ZnO sensor The biosensor was employed for uric acid determination in urine samples. The biosensor was stable upto 20 days with high thermal stability upto 85˚C (Zhang, *et al.,* 2004).

B. ZnO Micro/Nanowire Based Uric Acid Biosensor

An individual zinc oxide (ZnO) micro/nanowires based electrochemical uric acid biosensor was prepared. The wires were synthesized by chemical vapor deposition and possess uniform morphology and high crystallinity. Uricase was immobilized on the surface of the ZnO micro/nanowires by physical adsorption. The uric acid biosensor had a fast electron transfer between the active site of uricase and the surface of electrode. The biosensor showed high sensitivity (89.74 μA cm^{-2} mM^{-1}) and a wide linearity between 0.1 mM and 0.59 mM uric acid concentrations (Zhao, 2013).

C. *ZnO/GR Nanocomposite on Graphite Screen Printed Electrode*

An electrochemical sensor has been fabricated using ZnO/GR nanocomposite for selective determination of uric acid in a phosphate buffer solution (PBS, pH 7.0). The electrochemical behavior of uric acid at the ZnO/GR nanocomposite modified graphite screen printed electrodes (SPE) was studied by cyclic voltammetry and differential pulse voltammetry methods. The modified electrode exhibited remarkably anodic peak corresponding to the oxidation of uric acid over the concentration range of 1.0–100.0 μM with detection limit of 0.43 μM (*S/N* = 3). The fabricated sensor was further applied to the detection of uric acid in urine samples with good selectivity and high reproducibility (Rezaei *et al.*, 2018)

D. *ZnS quantum dots on gold disk electrode*

It was a reagent less amperometric uric acid biosensor based on ZnS quantum dot (QD). In this biosensor, *Arthrobactor globiformis* uricase was immobilized onto the carboxylic group functionalized ZnS QD by adsorption. The biosensor had following characteristics: ***Optimum pH:***6.9, ***Interference*** Little interference of ascorbic acid, ***Linearity*** 5.0×10^{-6} to 2.0×10^{-3} $molL^{-1}$, LOD: 2.0×10^{-6} $molL^{-1}$, ***Stability:*** Stable for 20 days. The biosensor was applied for determination of uric acid in blood (Zhang *et al.,* 2006).

E. *CHIT/Iron Oxide NPs/PANI/Pt.*

Uricase was immobilized covalently onto iron oxide nanoparticles/chitosangraft-polyaniline (Fe_3O_4-NPs/CHIT-*g*-PANI) composite electrodeposited onto Pt electrode. A uric acid biosensor was developed using uricase/Fe_3O_4-NPs/CHIT-*g*-PANI/Pt electrode as working electrode. The biosensor exhibited optimum response within 1s at pH 7.5 and 30 °C, when polarized at 0.4 V. There was a linearity between biosensor response (in current) and uric acid concentration ranging from 0.1 to 800 μM. The biosensor had a detection limit (LOD) of 0.1 μM (*S/N* = 3). The biosensor lost only 10% loss in its initial activity after its 120 uses during its storage at 4 °C for 100 days. The biosensor was employed for determination of uric acid in sera of apparently healthy persons. There was a good correlation between present biosensor and a standard enzymic colorimetric ($R^2 = 0.98$) (Devi and Pundir, 2013).

F. *AuNPs-rGO Film on ITO Coated Glass Plate*

A uric biosensor was developed by immobilizing uricase covalently, onto composite of AuNPs and rGO thin film electrodeposited onto ITO coated glass plate to construct UOx/Au-rGO/ITO sensor. The biosensor detected uric acid in sera in a wide range of uric acid concentration, 50-800 μM. The biosensor showed a detection limit of 7.32 ± 0.21 μM and a sensitivity of 86.62 ± 0.19 μA mM^{-1} by measuring differential pulse voltammetry (DPV) at low potential (0.228 V). The Km value for uric acid was 51.75 μM, which signified high enzyme kinetics at electrode surface with uric acid. The biosensor detected uric acid in sera. The biosensor had a good reproducibility and a shelf life of 6 months. AuNPs and rGO provided improved electron transfer from high electro-active molecules to electrode (Verma *et al*., 2019).

G. *Ag-Fe2O3@PANI/GC electrode*

A highly sensitive electrochemical uric acid sensor was developed based on PANI merged into a silver-doped iron oxide (Ag-Fe_2O_3) nanocomposite modified GC electrode.The nanocomposite modified electrode showed an exceptional electrolytic activity and reversibility to the oxidation of uric acid in a 0.1M phosphate buffer (pH7.0) compared to those in PANI and Ag-Fe_2O_3.The sensor showed a dynamic linearity in the range 0.001-0.900μM and LOD of 102pM for uric acid and good selectivity, reproducibility and longtime stability. The sensor was applied to measure uric acid in serum and urine with good analytic recoveries (Ponnaiah *et al*., 2018)

H. *Nanocomposite of Aunps, Hydroxyapatite (HAP) Nanowires, and Rgo on GC Electrode*

A ternary nanocomposite consisting of AuNPs, hydroxyapatite (HAP) nanowires, and rGO was synthesized by a simple one-step hydrothermal method, and electrodeposited onto GC electrode for detecting uric acid. The nanocomposite was characterized through various methods such as scanning electron microscopy (SEM), TEM, and X-ray diffraction. The electrochemical measurements of the modified GCE were carried out in a conventional three-electrode system. The experimental results showed that the obtained HAP nanowire and rGO were mixed homogeneously, and the AuNPs were deposited into this matrix. The GCE modified by these nanocomposites had superior electrocatalytic activities for uric acid. The peak current intensities of uricase/HAP-rGO/AuNPs electrode showed linear increase as the uric acid concentration increased ranging from 1.95×10^{-5} to 6.0×10^{-3} M ($R^2 = .9943$), with a LODof 3.9×10^{-6} M (S/N = 3) and analytical sensitivity of 13.86 mA/M. The biosensor measured uric acid level in human urine samples (Chen *et al*..,2020)

I. Hemin Functionalized GrO Nanosheet/GC Electrode

An electrochemical uric acid biosensor was developed using hemin functionalized grapheme oxide (GO) without any other reducing agents and the hemin functionalized graphene oxide (H-GO) hybrid nanomaterial of sonication, which could be ascribed to the π–π stacking and cation-π interactions between GO and hemin molecules. Due to the unique structure and excellent properties, H-GO modified GCelectrode showed high electrocatalytic activities for the oxidation of ascorbic acid, dopamine, and uric acid, thus providing the simultaneous determination for those biomolecules with LODs of 0.3, 0.17, and 0.17 μmol L^{-1} ($S/N = 3$), respectively. The biosensor showed high selectivity and good stability (Zou *et al.*.,2015)

2) Organic Nanomaterials Based Biosensors

A. Chitosan-Carbon Nano-Tube (CNT) Electrospun Nano Fiber on AgNPsAuE

An amperometric uric acid biosensor was developed by immobilizing uricase on an electro spun nano composite of chitosan-carbon nanotubes nanofiber (Chi–CNTsNF) covering an electrodeposited layer of silver nanoparticles (AgNPs) on a Au electrode (uricase/Chi–CNTsNF/AgNPs/Au). The biosensor showed optimum response at a potential of −0.35V vs Ag/AgCl in a flow-injection system based on the change of the reduction current for dissolved O_2 during oxidation of uric acid by the immobilized uricase. The biosensor response was directly proportional to the uric acid concentration. Under the optimum conditions, the biosensor had a very wide linear range, 1.0–400 μmol L^{-1}, with a very low detection limit of 1.0 μmol L^{-1} ($s/n = 3$). The operational stability of the uricase/Chi–CNTsNF/AgNPs/Au biosensor (up to 205 injections) was excellent and stored for >6 weeks. The biosensor had a low Km of 0.21 mmol L^{-1} indicating the high affinity of immobilized uricase for uric acid. The ascorbic acid, glucose, and lactic acid, had negligible effects on the activity of the biosensor. The biosensor measured uric acid level in sera, which correlated well with those by standard enzymatic colorimetric method ($P > 0.05$) (Numnuam *et al*., 2014).

B. MWCNTs/SnO_2NPs

A novel reagent less amperometric uric acid biosensor based on adsorption of *Arthrobactor globiformis* uricase onto functionalized multiwalled carbon nanotubes (MWCNTs) with tin oxide (SnO2) nanoparticles was made and applied to assay uric acid levels from an in vivo micro dialysis sampling. Compared with unfunctionalized or traditional carboxylic acid (–COOH) functionalized MWCNTs, the MWCNTs–SnO_2 electrode exhibited higher electrocatalytic oxidation to uric acid. The biosensor had following characteristics:

Optimum pH: 6.9, ***Linearity:*** 1.0×10^{-7} to 5.0×10^{-4} mol L^{-1} ***Interference:*** Little ascorbic acid interference. The high sensitivity of the MWCNTs–SnO_2 modified enzyme electrode enabled the monitoring of trace levels of uric acid in dialysate samples in rat striatum. ***Stability:*** The sensor was stable for 20 days. (Zhang *et al.,* 2005).

C. MWCNTs/AuNPs

An amperometric uric acid biosensor was fabricated by immobilizing uricase onto gold nanoparticle (AuNPs)/ MWCNT layer deposited on Au electrode via carbodiimide linkage. The sensor measured uric acid levels in sera of apparently healthy individuals and persons suffering from gout. A number of serum substances had practically no interference. The other characteristics of biosensor were as follow: ***Response time*****:** 7s ***Linearity*****:** 0.01–0.8 mM ***Detection limit*****:** 0.01 mM (Chauhan and Pundir, 2011)

D. MWCNT/AgNi/SPCE

Sihombing *et al.*. (2024) developed enhanced uric acid biosensor using SPCE functionalized with MWCNT/Ag Ni nanocomposite and compared them fabric-based biosensors. The functionalized f-MWCNT/AgNi was confirmed by UV-Vis, Raman specrtra, SEM, EDX Disforcive X-ray analysis and XRD analyses. The nanocomposite was deposited on working electrode (WE) surface by drop casting method. The current generated by uricase from uric acid was measured by CV and DPV. The DPV curves revealed sensitivities 2.769 μA /mM and 4.638μA/mM for SPCE and fabric-based electrodes and LOD were 0.024and 0.017 mM respectively and linearity were 0.05-1.00mM and 1.0-5.0 mM respectively for SPCE and fabric-based electrodes. Both biosensors were selective for uric acid in presence of ascorbic acid, glucose, lactic acid and ethanol.

E. PANI/MWCNT

An amperometric uric acid biosensor was constructed by immobilizing uricase onto PANI/MWCNT electrodeposited onto Indium tin oxide (ITO) coated glass plate by carbodamide linkage. The biosensor showed a very quick response within 8 sec with a minimum detection limit of 5 μM. The electrode system was stable upto180 days with slight loss of its enzyme activity. The biosensor was applied for quantitative analysis of uric acid level in sera of apparently healthy individuals and the persons suffering from hyperuricemia and gout (Bhambi *et al*., 2010).

F. MWCNT/PBNP/PANI

A uric acid biosensor was developed using uricase immobilized through glutaraldehyde coupling onto chitosan- onto Prussian blue nanoparticles (PBNPs) absorbed onto c-MWCNTs and PANI electrochemically deposited onto Au wire. The biosensor exhibited optimum response within 4s at pH 7.5 and 40°C, at 0.4V vs. Ag/AgCl. A linear working range was obtained for uric acid

concentration range, 0.005-0.8 mM, with a detection limit of 5 µM. The biosensor showed 96% recovery of added uric acid in sera and 4.6 & 5.4% within and between batch of coefficient of variation respectively and a good correlation (R^2=0.99) with standard enzymic colorimetric method. This sensor measured of uric acid in sera. It lost only 37% of its initial activity after its 400 uses over a period of 7 months, when stored at 4°C (Rawal *et al*., 2011).

3) Enzyme Nanoparticles Based Biosensor

We prepared nanoparticles (size =100 nm) of commercial uricase by desolvation method using ethanol as dehydrating agent and immobilized them directly onto polycrystalline Au electrode and used as working electrode (WE). This WE were characterized by SEM, FTIR and EIS. The electrode showed maximum current within 7s at pH 8.5 and $40^{0.}$ The electrode showed linearity for uric acid in the range 0.005-0.8mM with a LOD of 5.0 µM and sensitivity of 0.03mA/µM /cm2.The electrode /biosensor was applied for detection of uric acid in serum and urine. It showed a high stability in cold, as lost only 15% of its initial activity over a period of 7months, when stored in cold at 4°C (Chauhan *et al*., 2011).

H_2O_2 Independent Biosensors

Koch and Silinia (2024) detected uric acid by H_2O_2 independent biosensors i.e. no electrooxidation of uric acid , and application in model and fermentation samples.A novel amperometric enzymatic H_2O_2 independent sensing at low concentration (<50 µM)The uric acid sensitivity relied on the use of SPE modified by an electrodeposited hybrid functioning sensing film comprising a non-noble electrocatalyst, a bioorganic layer consisting uricase and data acquisition enabling the biochemical transformation of uric acid. The biosensor was selective for uric acid in presence of adeninine, xanthine, urea, ascorbic acid, ethanol and glycerol. The biosensor measured uric acid in microbial cells supernatant (*S. cerevisae* and *E. coli*) at low conc.(<50µM). Sensing hybrid was CuNPs/Uricase/Nafion layer on SPE/GrO. The method was based on O_2 consumption,which was measured by needle based O_2 minisensor.

Potentiometric Biosensor

The biosensor consists of an immobilized uricase membrane surrounding the probe from a pH-meter

Principle: Potentiometric biosensors are based on ion-selective electrodes (ISE) and ion-sensitive field effect transistors (ISFET). The primary outputting signal is possibly due to ions accumulated at the ion-selective membrane interface. Current flowing through the electrode is equal to or near zero (Pohanka and Skladal, 2008). The potentiometric biosensors are based on sensing of following reaction species:

(i) H^+ Producing reactions, (ii) H^+ Consuming reactions, (iii) $NH4^+$ Producing reactions, (iv) Anion producing reactions

Generally, three types of ion-selective electrodes are of use in biosensors:

(i) Glass electrodes for cations, (ii) Glass pH electrodes coated with a gas- permeable membrane selective for CO_2, NH_3 or H_2S. (iii) The iodide electrode is useful for the determination of I^- in the peroxidase reaction. The potentiometric biosensors are described below depending on the type of working electrode:

Tin Oxide (SnO2)/Indium Tin Oxide (ITO) Glass

A disposable potentiometric uric acid biosensor was constructed by co-immobilizing uricase, catalase, and electron mediator on the surface of the SnO_2/ITO glass. The SnO_2 / ITO glass was employed as a pH sensor, fabricated by sputtering SnO_2 thin films on the ITO glass. 3-Glycidyl-oxypropyl-trimethoxysilane (GPTS) was employed to immobilize uricase, catalase and the electron mediator (ferrocenecarboxylic acid, FcA) on the sensing window. The experimental results showed that the optimal weight ratio of uricase, FcA to catalase (CAT) was 4:1:2. The biosensor had following characteristics. ***Response time:*** 3.5 min, ***Linearity:*** 2 mg/dl and 7 mg/dl at pH 7.5, in 20 mM of test solution, with a correlation coefficient of 0.99213, ***Interference:*** No significant interference by interfering substances like, glucose, urea and ascorbic acid, ***Stability:*** The recorded voltage was relatively constant during the first 28 days of measurement. Consequently, a potentiometric uric acid biosensor was realized with the advantages of low cost and simple fabrication **(**Liao *et al.,* 2006).

Dodecyl Sulfate Doped Poly (N-Methylpyrrole)

A new type of electronically conducting polymer-based uric acid sensor was constructed by using dodecyl sulfate doped poly (N-methylpyrrole) for *Candida ulitis* uricase immobilization through adsorption. The results proved that this polymer film could incorporate the enzyme during its electrochemical preparation assumingly owing to the electrostatic interaction between the oxidized layer and the basic form of the enzyme. The lay-out prepared by thick film technology was designed for bipotentiostatic measurements, where the two working electrodes with and without the enzyme were analogously polarized. The biosensor had following characteristics: ***Optimum pH***: 7.0, ***Optimum temp:*** 30ºC, ***Linearity:*** 100- 900 mM, LOD: 100mM (Dobay *et al.,* 1999).

DNA-Modified Graphite Powder Microelectrodes

The powder microelectrode technique combined with DNA immobilization on the surface of graphite powder led to DNA-modified graphite powder microelectrodes

(DNA/GPMEs) which were used for selective and sensitive detection of uric acid in urine. The biosensor had following characteristics. ***Linearity:*** 1.0 × 10^{-7} to 5.0 × 10^{-5} mol/L, LOD= 5.0 × 10^{-8} mol/L ***Interference:*** Under optimal conditions (DNA/GPMEs) eliminated the interference of ascorbic acid (AA) and greatly improved the detection sensitivity. The biosensor was applied for determination of uric acid in urine without any pretreatment of samples, gave satisfactory results (Luo *et al.,* 2005).

Naflon /Ruthenium Oxide Pyrochlore Modified Electrode

A chemically modified electrode with Naflon /ruthenium oxide pyrochlore was prepared and used for the determination of uric acid. The biosensor had following characteristics. ***Linearity:*** 7.5× 10^{-5} to 5.0 × 10^{-7}M, LOD= 1.1× 10^{-} and no interference by ascorbic acid at less than 10 folds concentration of substrate the biosensor was employed in selective measurement of uric acid in serum and urine with a 99% recovery for urine and 93.4 % for serum (Zen, *et al.,* 2002).

Non-Enzymatic Uric Acid Sensor Using Off Chip Extended Gate Field Effect Transistor:

A potentiometric non-enzymatic sensor using off-chip extended-gate field effect transistor (EGFET) with a ferrocenyl-alkanethiol modified gold electrode was developed for determination of uric acid in human serum and urine. Hexacyanoferrate (II) and (III) ions were used as redox reagent. The sensor measured the interface potential on the ferrocene immobilized Au electrode, which was modulated by the redox reaction between uric acid and hexacyanoferrate ions. The device showed a near Nernstian response to uric acid and was highly specific. The sensor was unaffected by glucose, bilirubin, ascorbic acid and hemoglobin The sensor also exhibits excellent long-term reliability. This extended gate field effect transistor-based sensors can be used as a point of care uric acid testing tool, due to the small size, low cost, and low sample volume consumption (Guan *et al*.., 2014)

Nagal *et al.* (2023) synthesized puffy balls like CoO nanostructure and studied its CV and DPV. The DPV based sensor exhibited better selectivity, reproducibility and applicability in non-enzymic electrochemical analysis of uric acid in human blood.

Nanoporus Gold Electrode:

A potentiometric uric acid biosensor was fabricated using a miniaturized nanoporus gold electrode (NPG). The NPG electrode was prepared by attaching a nanoporus gold leaf to a modified gla capillary and establishing an electrode connection with copper tape.A linear dependence between open circuit potential (OCP) and log of uric acid concentration (Freeman *et al*., 2021)

Carbon Paste Electrode:

A reagentless uric acid biosensor by immobilizing uricase and horseradish peroxidise (HRP) in carbon paste without the addition of an electron transfer mediator was reported. The poly (O-aminophenol) was electropolymerized at the working electrode acting as a conducting polymer layer. The biosensor had following characteristics: ***Response time:*** 30s, ***Linearity:*** 3 × 10^{-6} M to 1.0 × 10^{-4}M, LOD:3 × 10^{-6} M (Miland *et al*., 1996)

A uric acid biosensor was fabricated by the Langmuir–Blodgett (LB) technique to immobilize the uricase on chitosan/Prussian blue (CS/PB) pre-functionalized indium-tin oxide (ITO) electrode. The effects of ionic strengths, acidity of subphase, and uricase amount on the film were studied. The electrochemical properties of the uricase/n-nonadecanoic acid (UOx/NA) LB film proved that CS/PB was a good electro-catalyst for the reduction of hydrogen peroxide produced by enzymatic reaction of UOx, and protein molecules retained their natural electro-catalytic activity. The other analytic characteristics were as follow: ***Response time:*** s, ***Linearity:*** 5_10_6 to1.15_10_3mol/L, LOD:1.8-10^{-7}mol/L(Wang*et al.*.2010)

Chemiluminescence Biosensor

Principle

Luminescent transitions of excited molecules or atoms to a state of lower energy are characterized by electromagnetic radiations dissipated as photons in the ultraviolet, visible, or near infrared region. These luminescent reactions are classified according to the energy source involved during the excitation step. Thus, the most classical light emission chemical reactions are referred as chemiluminescence. Electrogenerated luminescence sensors have been developed with the aim of combining the sensitivity of light-emitting reactions with the convenience of sensors (Marquette and Blum,2007) These electrochemiluminisce biosensor are described as follow:

Nickel (II) Tetrasulfophthalo-Cyanine (NiTSPc)/ITO Electrode

A film of Nickel (II) tetrasulfophthalo-cyanine (NiTSPc) has been electrodeposited onto ITO electrode to form an electro chemiluminescent (ECL) behavior of bis-[3,4,6-trichloro-2-(pentyloxy- carbonyl)-phenyl] oxalate (BTPPO) on this modified electrode. The emission of ECL of BTPPO was greatly enhanced by hydrogen peroxide at this modified electrode. Uricase was immobilized on this modified ITO electrode to form a new ECL biosensor for uric acid. The reaction of uricase produces H_2O_2 from uric acid. The biosensor had following characteristics: Linearity*:* 6.0 ×10^{-} ^{5} to6.0 ×10^{-4}M, LOD: 8×10^{-6}M, ***Interference:*** A slight interference by ascorbic acid (Lin *et al.*, 2008).

Chip Based Microreactor

A chemiluminecence based biosensor on a chip coupled to micro fluidics system and a micro reactor with sol-gel method, was developed with co-immobilizition of HRP and luminal in the micro reactor followed by immobilization of uricase in the enzymatic reactor.

The biosensor had following characteristics. ***Linearity:*** 1 to 100 mg/L, LOD= 0.1 mg/L, ***Stability:*** Stable for 4 days. The biosensor measured uric acid in serum with, (Lv *et al.,* 2002).

Chitosan Support Membrane, Polyurethane Entrapped Ferrocene Film

A new detection system based on microdialysis sampling and chemiluminescence (CL) reaction was developed for in vivo monitoring of uric acid with high sensitivity, selectivity and accuracy. The uric acid was indirectly monitored by CL detection of uricase reaction product formation (H_2O_2). A microprobe was modified and coated with immobilized enzyme through a Streptavidin–biotin mediated linker using a chitosan support membrane. Polyurethane trapped ferrocene film was employed to protect the probe surface and diminish the interference from reductant molecules found in the blood (e.g. ascorbic acid). The biosensor had following linearity=0.01–1mM with a LOD=5µM, the biosensor was employed in monitoring of uric acid variation in vivo through an acute myocardial infarction model with good agreement with those obtained by a standard method. The procedure was recommended for in vivo and real time monitoring of uric acid. (Yao *et al.,* 2003).

Optical Biosensor

Principle: The principle of optical biosensor is based on determining the changes in light absorption between the reactants and products of a reaction (use of colorimetric test strips), or measuring the light output by a luminescent process.

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

$$\text{Chromogen(2H)} + 2H_2O \xrightarrow{\text{Peroxidase}} \text{Dye(D} - \text{luciferin)} + 2H_2O$$

$$\text{ATP} + \text{D} - \text{luciferin} + O_2 \xrightarrow{\text{luciferase}} \text{Xyluciferin} + \text{AMP} + \text{pyrophosphate} + CO_2 \text{ light (562 nm)}$$

Optical biosensor for uric acid is divided further on the following transducers:

Metal Oxide Semiconductor (CMOS) Photo Array Sensor and Polymeric Enzyme Biochip

An improved uric acid biosensor was developed with an optical polymeric biochip system based on the complementary metal oxide semiconductor (CMOS) photo array sensor and polymeric enzyme biochip for rapidly quantitating uric acid in a one-step procedure. The CMOS sensor was designed with N+/P-well structure and fabricated using a standard 0.5mCMOS process. The polymeric enzyme biochip was immobilized with uricase–peroxidase. The reaction medium was filled with the reacting medium with the sample. This study encompassed the cloning of the *Bacillus subtilis* uricase gene and expression in *Escherichia coli*, as well as the purification of uricase and measurement of its activity. The biosensor had linearity between 2.5 to 12.5 mg/dl (Huang *et al.,* 2004).

Transparent Polymer Poly (Dimethylsiloxane)

A microsystem was designed especially for measurement of uric acid concentration in biological samples with the use of different optical detection methods: spectrophotometric and fluorescence. The construction of electrode was based on modules that were made of a transparent polymer poly(dimethylsiloxane) (PDMS). The biosensor showed linearity between 5.9×10^{-5} M to 2.14×10^{-4} (Grabowska *et. al.,* 2008)

Sol–Gel Fluorescent Biosensor

A simultaneous encapsulation of a coupled uricase (*B. fastodious*)–peroxidase (*Horseradish*) system and amplex red in a sol–gel matrix allowed to obtain a reagent-less and ready-to-use biosensor for the accurate detection of uric acid in highly diluted biological fluids. The biosensor had following characteristics. ***Linearity:*** 20nM to 1mM, LOD:20 nM. The high sensitivity of the biosensor permitted a reliable determination of uric acid concentrations in the presence of interfering species (e.g. ascorbic acid) just by sample dilution (up to 50,000 for urine and 10,000 for serum and blood). The sol–gel encapsulation preserved the hierarchy of the enzyme activity, as demonstrated by the performance of the fluorescent biosensor (Martinez-Perez, 2003). The biosensor quantified uric acid concentrations in sera of apparently healthy humans and persons suffering from gout. No interference by electroactive mixes, such as glucose, cholesterol, urea, pyruvate, bilirubin, CuSO4, KCl, FAD, NaCl, $ZnSO_4$, NADH, $CaCl_2$, EDTA, riboflavin, and $MnCl_2$ was observed. However, ascorbic acid showed interference at its physiological concentration.

Enzymatic Biosensors Dependent on Screen-Published Cathodes

The altered SPE had the capacity to apprehend the H_2O_2, delivered by the reaction of uricase from uric acid, get immobilized straightforwardly on the adjusted floor of the running anode. The uric acid in sera was determined in the range, 0.03 – 0.3 mM with a detection limit of 0.01mM. A SPE consolidating the electrocatalyst cobalt phthalocyanine into a water-based ink plan, was tested as a base transducer for a uric acid biosensor (Kanyong *et al*., 2012). A sandwich biosensor was synthetic by means of first storing cellulose acetic acid derivation in this transducer, trailed through uricase lastly through a polycarbonate layer (Fig. 2). The uric acid in urine was determined in the range,0.5 - 0.25 mM

with a sensitivity of 2.10 µA/mM, whilst the exactness determined on unspiked urine changed into 5.8%.

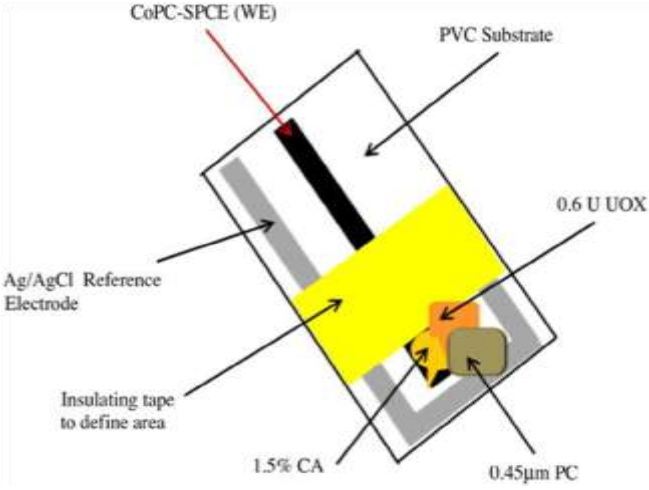

Fig. 2: Diagram of proposed sandwich biosensor strip: Cobalt phthalocyanine-SPE (CoPC–SPE) (working electrode, WE) and Ag/AgCl reference electrode; 1.5% cellulose acetate (CA); Uricase (UOx) enzyme, polycarbonate (PC) membrane over the enzyme layer (Perello *et al.*, 2005)

Langmuir– Blodgett Film-Based Terminals

Langmuir– Blodgett (LB) movies can be portrayed as profoundly sorted out extremely-meager natural movies, jogging from one to three many atomic layers. In LB strategy, a thick Langmuir monolayer is formed at the air–water interface. Langmuir monolayer was exchanged onto a strong substrate and multilayer movies are synthetic in this sturdy substrate by using the modern testimony of the monolayers. Langmuir– Blodgett (LB) films have the upsides of controllable thickness and confined lack of biomolecule movement. Special biosensors dependent on LB movies were created. Nevertheless, the usage of LB movies inside the territory of uric acid biosensors is confined. LB approach becomes utilized to immobilize uricase on chitosan/Prussian blue prefunctionalized ITO cathode for uric acid biosensor improvement. Any other examination reported the adsorption of uricase onto LB monolayers of stearic acid and the change of monolayers to strong backings as LB films. As LB films show guarantee for the improvement of biosensors, more accentuation need to receive to this gadget in uric acid biosensors (Zanon *et al.*., 2012).

SPR Based Uric Acid Biosensor

AuNPs/GCE

A sensitive and novel method for determining uric acid was developed, in which the glassy carbon electrode (GCE) was modified with electrodeposition Au nanoparticles and used to monitor the concentration of uric acid with the assistant of visible light illumination. A series of cyclic voltammetry (CV) and differential pulse voltammetry (DPV) measurements revealed that the introduction of visible light could greatly enhance both the strength and stability of response current due to the surface plasmon resonance (SPR). Specifically, the DPV showed a linear relationship between peak current and uric acid concentration in the range of 2.8 to 57.5 µM with the equation of I_{pa} (µA) = 0.0121c_{UA} (µM) + 0.3122 (R^2 = 0.9987). The visible light illuminated Au/GCE has a potential to be a sensitive electrochemical sensor in the future (Shi *et al.*.,2017)

GO/AuNPs-Coated Micro-Ball Optical Fiber Sensor

A highly sensitive and selective optical fiber-based enzymatic biosensor was developed for detection of uric acid in human serum. The working mechanism of sensor depends on surface plasma property and localized surface plasmon resonance technique. To achieve this aim, a micro-ball fiber sensor probe of 350 µm diameter was fabricated using advanced fusion-splicer and coated with AuNPs and GO to enhance its sensitivity. The sensor probe was functionalized with uricase for determination of uric acid. The linearity response of uricase/GO/AuNPs-coated micro-ball optical fiber sensor was in the range of 10 µM –1 mM uric acid concentration. The sensitivity of the sensor was 2.1 mM, with a LOD of 65.60 µM. The results of this sensor were correlated well with the results of A5800 Automatic Biochemical Analyzer. The sensor measured uric acid in human sera with 97.5% recovery (Kumar *et al*.2020).

Non Enzymic Uric Acid Sensors

Off Chip Extended Gate Field Effect Transistor

A potentiometric non-enzymatic sensor using off-chip extended-gate field effect transistor (EGFET) with a ferrocenyl-alkanethiol modified gold electrode was developed for determination of uric acid in human serum and urine. Hexacyanoferrate (II) and (III) ions were used as redox reagent. The sensor measured the interface potential on the ferrocene immobilized Au electrode, which was modulated by the redox reaction between uric acid and hexacyanoferrate ions. The device showed a near Nernstian response to uric acid and was highly specific. The sensor was unaffected by glucose, bilirubin, ascorbic acid and hemoglobin The sensor also exhibits excellent long-term reliability. This extended gate field effect transistor-based sensors can be used as a point of care uric acid testing tool, due to the small size, low cost, and low sample volume consumption (Guan *et al*., 2014)

CPE/LFONR

A quick, basic and delicate uric acid sensor was constructed through a nano gum. Lewatit FO36 nano gum, a macroporous, mono scattered, and pitifully critical, polystyrene-primarily based gum with graphite powder, as new modified carbon glue cathode (CPE/LFONR). The

electrochemical conduct of uric acid at CPE/LFONR was examined via cyclic voltammetry, instantly range voltammetry (LSV), chronoamperometry (CA) and chronocoulometry (CC). The sensor showed optimum response within 3s at pH 6.0 and linearity in the uric acid concentration range, 3.1 to 32.0µmol/ L with a LOD of 0.176µmol/ L. The sensor was employed for determination of uric acid in human urine and blood serum (Rajabi *et al*., 2017)

rGO/PDA/AuNPs

A uric acid sensor was developed using nanocomposites of rGO/PDA, prepared by reduction of GO nanosheets with dopamine. AuNPs were also attached by in situ reduction onto RGO/PDA to form RGO/PDA/AuNPs nanocatalyst and used for electrochemical analysis. This sensor detected three molecules, ascorbic acid, dopamine and uric acid simultaneously. The LOD for uric acid was 0.13 mM, this nanocatalyst provided good biocompatibility and large surface area with high conductivity (Shi *et al*., 2019)

3D MoS_2-PANI/rGO-Sensor

MoS_2 nanosphere with PANI were kept on rGO to make a three-dimensional structure (3D MoS_2-PANI/rGO) for detection of uric acid along with ascorbic acid and dopamine. The 3D MoS_2-PANI/rGO nanocomposite provided better electrochemical activity, functionality and bioaffinity also. The sensor detected uric acid with a detection limit of 0.36 µM with a linear range of 1.0 to 500 µM via DPV. This 3D MoS_2-PANI/rGO-sensor offered good reliability and stability for detection of these three biomolecules (Li et. al., 2019).

MWCNT with Porous g-C_3N_4

A sensor was developed using MWCNT with porous g-C_3N_4 (PCN) for uric acid determination in serum. This combination increased the redox peak values of uric acid and made the sensor more sensitive with a detection limit of 0.139 µM (Lv et. al., 2019).

MoS_2 Microflower

While all reports on electrochemical uric acid sensors are on rigid electrodes and based on either complex fabrication procedures or multiple steps-based synthesis techniques, this paper is the first demonstration of a single step hydrothermally grown MoS_2 on Al foil based flexible electrochemical sensor for non-enzymatic detection of UA. FESEM images revealed MoS_2 micro-flower like structure comprising of interlaced nanosheets, while chemical characterization data confirmed the successful growth of few layered (>4 layers) MoS_2 on Al foil. The as-fabricated flexible sensor exhibited a limit of detection of 1.169 µM, a response time of <3 s, excellent reproducibility, selectivity towards uric acid over glucose, ascorbic acid, and urea with a sensitivity of 98.3 ± 1 nAµM^{-1} ($R^2 = 0.999$) in the dynamic range of 10–400 µM. This enhanced sensing ability can be attributed to high surface area of MoS_2, more numbers of active sites resulting from large numbers of defects and edges and higher proportion of metallic 1 T phase than semiconducting 2H phase. The sensor was also successfully evaluated for the detection of uric acid in a non-invasive sample like human urine and the data corroborates well with the uric acid concentration obtained from conventional biochemical tests performed in clinical laboratory. This highly sensitive, ultra-low-cost MoS_2 based flexible electrochemical sensor paved way for the development of affordable lab on chip devices for a wide range of bioanalytical applications (Sha *et al*., 2019).

Heme, $Fc(Cys)_2$ and Fc-ECG/GC

A uric acid sensor was also fabricated using a heme, $Fc(Cys)_2$ and Fc-ECG electro-deposited onto GC electrode to detect uric acid, dopamine and ascorbic acid simultaneously. The heme/GCE combination provided a good electro-catalytic capability, towards their detection The sensor showed a linearity between uric acid concentration ranging from 2.5 to 20 μmol/L with a LOD of 0.63 µmol/L The sensor was applied to detect uric acid in real samples. (Feng *et al*., 2020).

rGO/Pd@PPy NPs/GC

A non-enzymic sensor was constructed by electro-depositing a composite of PdNPs and PPy-rGO onto GC electrode to form rGO/Pd@PPy NPs/GC. The sensor detected ascorbic acid, dopamine and uric acid simultaneously. The composites of rGO/Pd@PPy NPs provided superior catalytic activity and conductivity for the detection of these molecules with higher current and oxidation peak intensities. The sensor detected uric acid as low as 4.7×10^{-8} M with a linear range of 1×10^{-3} to 1.5×10^{-2} M (Demirkan *et al*., 2020).

Citric Acid-Fe_3O_4 NPs/GC

The nanoparticles of iron oxide iron (II, III) oxide (magnetite, Fe_3O_4) were precipitated with ammonium hydroxide and citric acid as a surfactant. A uric acid sensor was fabricated by modifying a GC electrode with citric acid-Fe_3O_4 NPs. This sensor detected uric acid as low as 7.5 μM with a linear range of 7.5 μM-0.18 mM. Citric acid-Fe_3O_4 NPs provided large surface area and hence showed enhanced catalytic activity (Sundar *et al*., 2020).

SPR Based Biosensor

Smartphone As an Electrochemical Analyser

The world's first medical smartphone was reported as an electrochemical analyzer, which is incorporated with an enzymatic test strip for rapid characterization of uric acid in peripheral whole blood. A disposable electrochemical uric acid test strip was connected to the electrochemical module integrated with smartphone through a specific interface, a slot around the edge of smartphone. A human finger whole blood drop (3µL) was applied on the strip for uric acid evaluation and compared to clinical biochemical analyzer

with satisfactory agreement. The measured data is saved and uploaded into personal health management center through the mobile Internet (GuO, 2016).

CONCLUSIONS

In conclusion, it can be deduced that electrochemical biosensors are better are better than conventional methods for quantitative determination of uric acid in biological fluids like titrimetry, colourimetry, enzymic-UV spectrometric, colourimetric & spectrofluorometric, chemiluminiscence, HPLC, Oscillating reaction methods, capillary electrophoresis, isotope dilution and radio-mass fragementography, as these are simple, specific, highly sensitive, reproducible ,accurate and provide rapid results against analyte of interest. Uric acid biosensors are employed for in vivo and real time monitoring of uric acid in blood and urine for diagnosis and treatment of gout, kidney, various intestinal diseases like uricolysis, cardiovascular diseases specifically acute myocardial infarction and in biofluids of sweat and wound healing and diurnal variations in salivary uric acid. The future research could be focused on developing and designing lab on paper chip and electronic chips to develop a economic and fully automatic device which can be used at the bedside of patients. The efforts can also made to miniaturize the laboratory model of uric acid biosensors with chip designing companies to provide the cheap portable device for monitoring the uric acid in biological fluids outside the laboratory.

Authors' Contribution

CSP: Conception, design, final checking and updating and discussion. SK: Literature collection and drafting of manuscript. Ravina: Assistance in preparation of the manuscript SP: Final writing and critical checking of manuscript. Final form of manuscript was approved by all authors.

Conflict of Interest

Authors declare no conflict of interest with the present publication.

REFERENCES

Arora J, Nandwani S, Bhambi M and Pundir CS (2009) Fabrication of dissolved O_2 metric uric acid biosensor using uricase epoxy resin biocomposite membrane. *Anal Chim Acta* **647:** 195–201. DOI: 10.1016/j.aca.2009.06.001

Arora K, Sumana G, Saxena V, Gupta RK, Gupta SK, Yakhmi, JV, Pandey MK, Chand S and Malhotra BD (2007) Improved performance of polyaniline-uricase biosensor *Anal. Chim. Acta* **594**: 17–23. DOI: 10.1016/j.aca.2007.04.068

Arslan F (2008) An amperometric biosensor for uric acid determination prepared from uricase immobilized in polyaniline-polypyrrole film. *Sensors*, **8**: 5492-5500. DOI: 10.3390/s8095492

Arya SK, Saha S, Ramirez-Vick JE, Gupta V, Bhansali S and Singh SP (2012) Recent advances in ZnO nanostructures and thin films for biosensor applications: review. *Anal. Chim. Acta* **737:** 1–21. DOI: 10.1016/j.aca.2012.05.048

Bardeletti G, Sechaud F and Coulet PR (1991) Amperometric enzyme electrodes forsubstrate and enzyme activity determinations. Cited in: Blum LJ and Coulet PR (ed). Biosensor principles and applications, Marcel Dekker, Inc. New York.

Bhambi M, Sumana G, Malhotra BD, Pundir CS (2010) An amperometric uric acid biosensor based on immobilization of uricase onto PANI-MWCNT composite film. *Art Cells Blood Subst Biotech* **38:** 178-185. DOI: 10.3109/10731191003716344

Bhargava AK, Lal H and Pundir CS (1999) Discrete analysis of serum uric acid with immobilized uricase and peroxidase. *J Biochem Biophys Methods* 39: 125-136. DOI: 10.1016/S0165-022X(99)00007-X

Bhargava AK and Pundir CS (2012) Determination of serum uric acid with arylamide glass bound uricase and peroxidase. *Intl J Adv Biotechnol Res* **2:** 307-317.

Bhawna and Pundir CS (2010) Fabrication of DO metric uric acid biosensor based on uricase bound to PVC membrane. *J Sci Indus Res* **69:** 695-699.

Buerk DG (1993) *Biosensors Theory and Applications,* Technomic Publishing, Pennsylvania U.S.A.

Bulger HA and Johns HE (1941) The determination of plasma uric acid. *J Biol Chem* **140:** 427–440. DOI: 10.1016/S0021-9258(18)51332-6

Castillo–Ortega MM, Rodriguez DE, Encinas JC, Plascencia M, Mendz FA and Olayo R (2002) Conductometric uric acid and urea biosensor prepared from electroconductive polyaniline Poly(n-butyl methacrylate) composites. *Sens Actuat B: Chem* **85**: 19-25. DOI: 10.1016/S0925-4005(02)00045-X

Cete S, Yasar A and Arslan F (2006) An amperometric biosensor for uric acid determination prepared from uricase immobilized in polypyrrole film. *Artif CellsBlood Substitut. Immobil Biotechnol.* **34:** 367-380. DOI: 10.1080/10731190600684116

Chauhan N and Pundir CS (2011) An amperometric uric acid biosensor based on MWCNT-AuNP composite. *Anal. Biochem.* **413**: 97-103**.** DOI: 10.1016/j.ab.2011.02.007

Chauhan N, Kumar A and Pundir CS (2014) Construction of an uricase nanoparticles modified gold electrode for amperometric determination of uric acid. *Appl Biochem Biotechnol* **174**(4):1683-1694. DOI: 10.1007/s12010-014-1097-6

Chauhan N, Preeti, Pinky and Pundir CS (2014) Covalent immobilization of uricase inside a plastic vial for uric acid determination in serum and urine. *Anal. Sci*. **30:** 501-506. DOI: 10.2116/analsci.30.501

Chen JC, Chung HH, Hsu CT Tsai DM, Kumar AS and Zen JH (2005) A disposable single use electrochemical sensorfor the detection of uric acid on human whole blood. *Sens Actuat B: Chem* **110**: 364-369. DOI: 10.1016/j.snb.2005.02.026

Chen XB, Calder AG, Prasitkusol P, Kyle DJ and Jayasuriya MC (1998) Determination of 15N isotopic enrichment and concentrations of allantoin and uric acid in urine by gas chromatography/mass spectrometry. *J Mass Spectrom* **33:** 130-137. DOI: 10.1002/(SICI)1096-9888(199802)33:2<130::AID-JMS616>3.3.CO;2-P

Chen Y, Zhou W, Ma J, Ruan F, Qi X and Cai Y (2020) Potential of a sensitive uric acid biosensor fabricated using hydroxyapatite nanowire/reduced graphene oxide/gold nanoparticle. *Microsco Res Techniq* **83**: 268-275. DOI: 10.1002/jemt.23410

Chen Z, Fang C, Wang H, He J and Deng Z (2008) A novel disposable amperometric UA strip *Sens Actuators B: Chem* **129**: 710–715. DOI: 10.1016/j.snb.2007.09.061

Cheng C, Kao C-Y (2015) An electrochemical biosensor with uricase on functionalized gold coated copper electrode for urinary uric acid assay *Electroanal* **28**: 695-703. DOI: 10.1002/elan.201500539

Conroy PJ, Hearty S, Leonard PO and Kennedy RJ (2009) Antibody production, design and use for biosensors-based applications. *Semin. Cell Dev Biol* **20:**10–26. DOI: 10.1016/j.semcdb.2009.01.010

Cooper N, Khosravan R, Erdmann C, Fiene J and Lee JW (2006) Quantification of uric acid, xanthine and hypoxanthine in human serum by HPLC for pharmacodynamic studies. *J Chromatogr B Analyt Technol Biomed Life Sci* **837:** 1-10. DOI: 10.1016/j.jchromb.2006.02.060

Cunningham AJ (1999). *Introduction to bioanalytical sensors*. 4th edition, New York, Wiley pp. 23-135

Dai X, Fang X, Zhang C, Xu RB and Xu J (2007) Determination of serum uric acid using high-performance liquid chromatography (HPLC)/isotope dilution mass spectrometry (ID-MS) as a candidate reference method. *J Chromatogr B* **857:** 287–295. DOI: 10.1016/j.jchromb.2007.07.035

Devi R and Pundir CS (2013) Construction and application of an amperometric uric acid biosensor based on covalent immobilization of uricase on iron oxide nanoparticles/chitosan-g-polyaniline composite film electrodeposited on Pt electrode. *Sens Actuat: B Chem* **193**: 608-615. DOI: 10.1016/j.snb.2013.12.010

Dinckaya E, Akyilmaz E and Akgol S (2000) Urate oxidase electrode based on dissolved oxygen probe for urine uric acid determination. *Indian J Biochem Biophys* **37**: 67-70.

Dobay R, Harsányi G and Visy C (1999) Detection of uric acid with a new type ofconducting polymer-based enzymatic sensor by bipotentiostatic technique. *Anal Chim Acta* **385**: 187-194. DOI: 10.1016/S0003-2670(98)00662-X

Ellerbe P, Cohen A, Welch MJ and White E (1990) Determination of serum uric acid by isotope dilution mass spectrometry as a new candidate definitive method. *Anal Hem* **62:** 2173-2177. DOI: 10.1021/ac00219a004

Falasca GF (2006) Metabolic diseases: gout. *CLIN Dermatol* **24:** 498–508. DOI: 10.1016/j.clindermatol.2006.07.015

Fang J and Alderman MH (2000) Serum uric acid and cardiovascular mortality the NHANES I epidemiologic follow-up study, 1971-1992. National Health and Nutrition Examination Survey. *JAMA* **283**: 2404–2410. DOI: 10.1001/jama.283.18.2404

Folin O (1930) An improved method for the determination of uric acid in blood. *J Biol Chem* **54:** 179-187. DOI: 10.1016/S0021-9258(18)76917-2

Fossati P, Prencipe L and Berti G (1980) Use of 3,5-dichloro-2-hydroxybenzenesulfonic acid/4-aminophenazone chromogenic system in direct enzymic assay of uric acid in serum and urine. *Clin Chem* **26:** 227-231. DOI: 10.1093/clinchem/26.2.227

Freeman CJ, Islam MS and Collinson MM (2021) Potentiometric biosensing of ascorbic acid, uric acid and cysteine in microliter volumes using miniaturized nanoporus gold electrode.2021 *Biosensors* **11**(1):10. DOI: 10.3390/bios11010010

Gagliardi ACM, Miname MH and Santos RD (2009) Uric acid: A marker of increased cardiovascular risk. *Atherosclerosis* **202**: 11–17. DOI: 10.1016/j.atherosclerosis.2008.05.022

Galban J, Andreu Y, Almenara MJ, Marcos S and Castillo JR (2001) Direct determination of uric acid in serum by a fluorometric enzymatic method based on uricase. *Talanta* **54**: 847–854. DOI: 10.1016/S0039-9140(01)00335-6

George SK, Dipu MT, Mehra UR, Singh P, Verma AK and Ramgaokar JS (2006) Improved HPLC method for the simultaneous determination of allantoin, uric acid and creatinine in cattle urine. *J Chromatogr B Analyt Technol Biomed Life Sci* **832:** 134-137. DOI: 10.1016/j.jchromb.2005.10.051

Grabowaska I, Stadnik D, Chudy M, Dybko A and Brzozka Z (2007) Architecture and method of fabrication PDMS system for uric acid determination. *Sens Actuators B: Chem* **121:** 445-451. DOI: 10.1016/j.snb.2006.04.101

Grabowska M, Chudy A, Dybko Z and Brzozka (2008) Uric acid determination in a miniaturized flow system with dual optical detection. *Sens Actuat B: Chem* **130:** 508–513. DOI: 10.1016/j.snb.2007.09.051

Guan W, Duan X and Reed MA (2014) Highly specific and sensitive non-enzymatic determination of uric acid in serum and urine by extended gate field effect transistor sensors. *Biosensors Bioelectronics* **51**: 225-231. DOI: 10.1016/j.bios.2013.07.061

Guang Pu, Xue Yu, Guang Pu and Fen Xi (2005) A novel chemiluminiscence method for determination of uric acid based on its inhibitory activity. **25:** 1213-1528.

Han SH, Ha YH *et al.* (2022) Electrochemical detection of uric acid in undiluted human saliva using paper integrating electrode. *Scientific Reports* **12**: 12033. DOI: 10.1038/s41598-022-16176-5

He D, Zhang Z, Huang Y, Hu Y, Zhou H and Chen D (2005) Chemiluminescence microflow injection analysis system on a chip for the determination of uric acid without enzyme. *Luminescence* **20**: 271–275. DOI: 10.1002/bio.847

Huang SH, Shih YC, Wu CY, Yuan CJ and Yang YS (2004) Detection of serum uric acid using the optical polymeric enzyme biochip system. *Biosens Bioelectron* **19**: 1627-33. DOI: 10.1016/j.bios.2003.12.026

Inoue K, Namiki T, Iwasky Y, Yoishimura Y and Nakazawa H (2008) *J Chromatogr B: Analyst Technol Biomed Life* **785:** 57-63. DOI: 10.1016/S1570-0232(02)00850-4

Jamde CV *et al* (2025) Enhanced uric acid detection using functionalized multi-walled/silver nickel oxide nano-composite :A comparative study on PCE and fabric based biosensing. *Sens Actuat Reports* **8**: 100223. DOI: 10.1016/j.snr.2024.100223

Jiang Y, Wang A, Kan J (2007) Selective uricase biosensor based on polyaniline synthesized in ionic liquid *Sens Actuat B: Chem* **124**: 529-534. DOI: 10.1016/j.snb.2017.06.146

Jindal K, Tomar M, Gupta VA (2017) Novel low-powered uric acid biosensor based on arrayed pn junction heterostructures of ZnO thin film and CuO microclusters. *Sens Actuat B: Chem* **253:** 566-75

Jossa F, Farinaro E, Panico S, Krogh V, Celentano E, Galasso R, Mancini M and Trevisan M (1994) Serum uric acid and hypertension: the Olivetti heart study. *J Hum, Hyperten* **8**: 677–681.

Kamoun P and Lafourcade JH (1976) Ultramicro method for determination of plasma uric acid *Clin Chem* **22:** 964-967.

Kan J, Pan X, Chen C (2004) Polyaniline–uricase biosensor prepared with template process. *Biosens Bioelectron* **19**: 1635–1640. DOI: 10.1016/j.bios.2003.12.032

Kanyong P, Pemberton RM, Jackson SKand Hart JP (2012) Development of a sandwich format, amperometric screen-printed uric acid biosensor for urine analysis. *Anal Biochem* **428**: 39–43. DOI: 10.1016/j.ab.2012.05.027

Koch MSilina YE (2024) Uric acid detection by hydrogen peroxide independent biosensors: Novel insight and application *Microchem J* **207**:112099. DOI: 10.1016/j.microc.2024.112091

Kudo H and Takagi Y (2018) Electrochemical biosensor for simplified determination of salivary uric acid *Sens Matr* **30** ;1187-1195. DOI: 10.18494/SAM.2018.1886

Kumar S *et al*. (2020) Development of uric acid biosensor using gold nanoparticles and graphene oxide functionalized micro-ball fiber sensor probe," in *IEEE Transactions on NanoBioscience* **19**: 173-182. DOI: 10.1109/TNB.2019.2958891

Kuwabata IS, Nakminami TS and Yoneyama H, (1999) A biomimetic phospholipid alkanethiolate bilayer immobilizing uricase and an electron mediator on an Auelectrode for amperometric determination of uric acid *Anal Chem* **71**: 4278-4282. DOI: 10.1021/ac981371p

Lai SVH, Kao P, Tadigadapa S (2011) Thermal biosensors from micromachined bulk acoustic wave resonators *Proc Eng* 25:1381–1384. DOI: 10.1016/j.proeng.2011.12.341

Li S, Ma Y, Liu Y, Xin G, Wang M, Zhang Z and Liu Z (2019) Electrochemical sensor based on a three dimensional nanostructured MoS 2 nanosphere-PANI/reduced graphene oxide composite for simultaneous detection of ascorbic acid, dopamine, and uric acid *RSC Adv* **9**: 2997-3003. DOI: 10.1039/C8RA09511F

Liang Y, Wang A , Kan l (2007) Selective uricase biosensor based on polyaniline synthesized in ionic liquid *Sens Actuat B:Chem* **124**:529-534. DOI: 10.1016/j.snb.2007.01.016

Liao CW, Chou TC, Sun PT, Hsing SK, Hcieh HJ (2006) Preliminary investigation on a new disposable potentiometric biosensor for uric acid *Anal Sci* **24**: 1435-30

Lin Z, Chen Z, Liu Y, Wang J and Chen G (2008) Anelectrochemiluminescent biosensor for uric acid based on the electrochemiluminescenceofbis-[3,4,6-trichloro-2-(pentyloxycarbonyl)-phenyl] oxalate on an ITO electrode modified by an electropolymerized nickel phthalocyanine film *Analyst*, **133:** 797 – 801. DOI: 10.1039/b716281b

Lohsoonthorn V, Dhanamun B, Williams MA (2006) Prevalence of hyperuricemia and its relationship with metabolic syndrome inThai adults receiving annual health exams. *Arch Med Res* **37**: 883–889. DOI: 10.1016/j.arcmed.2006.03.008

Lu X, Xu X, Yan X, Li S, Ren W and Cheng Z (2011) Piezoelectric biosensor based on ZnO micro membrane *Curr Appl Phys* **11:** S285–S287. DOI: 10.1016/j.cap.2011.03.006

Luo, JW, Zhang,M, Pang, DW, 2005 Selective and sensitive determination of uric acid at DNA modified graphite powder *Sens Actuat B: Chem* **106:** 358-362. DOI: 10.1016/j.snb.2004.08.020

Luo Y-C, Do J-S, Liu C-C (2006) An amperometric uric acid biosensor based on modified Ir-C electrode*BiosensBioelectron* **22**: 482-488. DOI: 10.1016/j.bios.2006.07.013

Lv J, Li C, Feng S, Chen SM, Ding Y, Chen C, Hao Q, Yang TH and Lei W (2019) A novel electrochemical sensor for uric acid detection based on PCN/MWCNT *Ionics* **25:** 4437-4445. DOI: 10.1007/s11581-019-03010-8

Lv Y, Zhang Z, Chen F (2002) Chemiluminescence biosensor chip based on amicroreactor using carrier air flow for determination of uric acid in human serum *Analyst* **127**: 1176-1179. DOI: 10.1039/b203501b

Marli M, Filho UP, Osvaldo NO, Ferreira M (2007) Immobilization of uricase in layer by layer films used in amperometric biosensor for uric acid. *J Solid State Electrochem* **11**: 1489-1495. DOI: 10.1007/s10008-007-0314-4

Marquette CA and Blum LJ (2007) Electro chemiluminiscent biosensing. *Anal Bioanal Chem* **390**: 155-168. DOI: 10.1007/s00216-007-1631-2

Mehrvar M and Abdi M (2004) Recent developments,characteristics and potential applications of electrochemical biosensors *Anal Sci* **20:** 1113–1125. DOI: 10.2116/analsci.20.1113

Merriman TR and Dalbeth N (2011) The genetic basis of hyperuricemia and gout. *Joint Bone Spine* **78:** 35–40. DOI: 10.1016/j.jbspin.2010.02.027

Miland E, Miranda AJ, Smyth MR and Fagain, CO (2010) Poly(o-aminophenyl) modified bienzyme carbon paste electrode for detection of uric acid *Talanta,*43(5),785-96. DOI: 10.1016/0039-9140(95)01825-5

Nagal V *et al* (2023) Highly sensitive non enzymatic uric acid sensor based on cobalt oxide puffi balls –like nanostructure *Biosensensors* (Basel) **12-13**(3): 375. DOI: 10.3390/bios13030375

Nakagawa T, Kang D-H, Feig D, Sanchez-Lozada LG, Srinivas TR, Sautin Y, Ejaz AA, Segal M and Johnson RJ (2006) Unearthing uric acid: An ancient factor with recently found significance in renal and cardiovascular disease. *Kidney Intl* **69**:1722–1725. DOI: 10.1038/sj.ki.5000391

Nanjo M and Guilbault GG (1974) Enzyme electrode sensing oxygen for uric acid in serum and urine. *Anal Chem* **46**: 1769–1772. DOI: 10.1021/ac60348a058

Numnuam A, Thavarungkul P and Kanatharana P (2014) An amperometric uric acid biosensor based on chitosan-carbon nanotubes electro spun nano fiber on silver nanoparticles. *Anal Bioanal Chem* **406:** 3763-3772. DOI: 10.1007/s00216-014-7770-3

Nyhan WLJ (1997) The recognition of Lesch-Nyhan syndrome as an inborn error of purine metabolism. *Metab Dis* **20**: 171–178. DOI: 10.1023/A:1005348504512

Odo J *et al* (2004) Spectrofluorometric determination of uric acid in serum and glucose by use of Fe(III)thiacalix (4)arenetetrasulfonate as peroxidase mimic. *J Health Science* **50**(6): 594-599. DOI: 10.1248/jhs.50.594

Pachla LA, Slaunwhite WD, Wenke DC and Kissinger PT **(**1975) Colorimetric, enzymatic, and liquid-chromatographic methods for serum uric acid. *Clin Chem* **21:**1427-1429. DOI: 10.1093/clinchem/21.10.1427

Pan X, Zhou S, Chen C and Kan A (2006) Preparation and properties of an uricase biosensor based on co-polymers of O-aminophenol aniline. *Sens Actuat B: Chem* **113**: 334-339. DOI: 10.1016/j.snb.2005.03.086

Perello J, Sanchis P and Grases F (2005) Determination of uric acid in urine, saliva and calcium oxalate renal calculi by HPLC/MS *J Chromatogr B* **824:** 175-180. DOI: 10.1016/j.jchromb.2005.07.024

Perello, J, Sanchis, P, Grases, F, (2005) Determination of uric acid in urine, saliva and calcium oxalate renal calculi by HPLC/MS. *J Chromatogr B* **824:**175-180. DOI: 10.1016/j.jchromb.2005.07.024

Pesce MA, Bodourian SH, and Nicholson JF (1974) Automated enzymatic micromethod for determination of uric acid in serum and urine with a centrifugal analyzer *Clin Chem* **20**: 1231-1233. DOI: 10.1093/clinchem/20.9.1231

Phaugat K, Bhambi M, Renu and Pundir CS (2010) Polyethylene terephthalate membrane as a support for covalent immobilization of uricase and its application in serum urate determination. *J Mol Catal B* **62:** 27–31. DOI: 10.1016/j.molcatb.2009.08.012

Pohanka M and Skladal P (2008) Electrochemical biosensors principles and applications. *J Appl Biomed* **6**:57-64. DOI: 10.32725/jab.2008.008

Ponnaiah SK, Periakaruppan P and Vellaichamy B (2018) New electrochemical sensor based on a silver-doped iron oxide nanocomposite coupled with polyaniline and its sensing application for picomolar-level detection of uric acid in human blood and urine samples. *The J Phys Chem B* **122**: 3037-3046. DOI: 10.1021/acs.jpcb.7b11504

Pundir CS and Bhargava AK (2003) Quantification of urinary urate with alkylamine glass-bound uricase and arylamine glass-bound peroxidase. *Indian J Clin Biochem* **18:** 22-28. DOI: 10.1007/BF02867661

Raj RC and Ohsaka T (2003) Voltammetric detection of uric acid in presence of ascorbic acid at a gold electrode modified with a self-assembled monolayer of heteroaromatic thiol. *J Electroanal Chem* **540:** 69–77. DOI: 10.1016/S0022-0728(02)01285-8

Rajabi H, Noroozifar M and Sabbaghi N (2017) Electrochemical determination of uric acid usingnano resin modified carbon paste electrode as a new sensor. *J Mater Appl Sci* **1**: 1002.

Rawal R, Chawla S, Chauhan N, Dahiya T and Pundir CS (2011) Construction of amperometric uric acid biosensor based on uricase immobilized on PBNPs/c-MWCNT/PANI/Au composite. *Intl J Biol Macromol* **50**: 112-118. DOI: 10.1016/j.ijbiomac.2011.10.002

Rezaei R, Foroughi MM, Beitollahi H *et al* (2018) Electrochemical sensing of uric acid using a ZnO/Graphene nanocomposite modified graphite screen printed electrode. *Russ J Electrochem* **54**: 860–866. DOI: 10.1134/S1023193518130347

Rocha DL and Rocha FRP (2010) A flow-based procedure with solenoid micro pumps for spectrophotometric determination of uric acid in urine. *Microchem J* **94:** 53–59. DOI: 10.1016/j.microc.2009.08.010

RoyChoudhury S, Umasankar Y, Hutcheson JD, Lev-Tov H, Kirsner R, & Bhansali S 2018 Uricase based enzymatic biosensor for non-invasive detection of uric acid by entrapment in PVA-SbQ polymer matrix. *Electroanalysis* **30***:* 2374-2385. DOI: 10.1002/elan.201800360

Sha R, Vishnu N, Badhulika S (2019) MoS_2 based ultra-low-cost, flexible, non-enzymatic and non-invasive electrochemical sensor for highly selective detection of uric acid in human urine samples. *Sens Actuat B: Chem* **279:** 53-60. DOI: 10.1016/j.snb.2018.09.106

Shi Y, Wang J, Li S et *al* (2017) The enhanced photo-electrochemical detection of uric acid on Au Nanoparticles modified glassy carbon

electrode. *Nanoscale Res Lett* **12:** 455. DOI: 10.1186/s11671-017-2225-3

Shi L, Wang Z, Chen X, Yang G and Liu W (2019) Reduced graphene oxide/polydopamine/gold electrode as elecrochemical sensor for simultaneous determination of ascorbic acid, dopamine, and uric acid. *Intl J Electrochem Sci* **14**: 8882-8891. DOI: 10.20964/2019.09.49

Sihombing YA *et al* (2024) Enhanced uric acid detection using functionalized multi-walled/silver nickel oxide nano-composite :A comparative study on PCE and fabric based biosensing. *Sens Actuat Reports* **8**: 100223. DOI: 10.1016/j.snr.2024.100223

Song Z-H and Hou S (2002) Chemiluminiscence assay of uric acid in human serum and urine using flow injection with immobilized reagent technology. *Anal Bioanal Chem* **372:** 327-332. DOI: 10.1007/s00216-001-1122-9

Steel AE (1963) A comparison of eleven methods for determination of serum uric acid. *Proc Assoc Clin Biochem* **2:** 211-215. DOI: 10.1177/036985646300200808

Sushma, Rekha, Kumar V, Suman and Pundir CS (2003) Determination of serum and urinary urate with reusable uricase strip. *Indian J Chemical Technol* **11:** 764-768.

Thakur B and Sawant SN (2013) Polyaniline/Prussian-Blue-Based Amperometric Biosensor for Detection of Uric Acid. *Chem Plus Chem* **78**(2): 166-74. DOI: 10.1002/cplu.201200222

Thomasson CG, Blijenberg BG and Leijnse B (1982) Evaluation of the Technicon bound uricase method for the determination of uric acid in urine. *J Clin Chem Clin Biochem* **20:** 299-303. DOI: 10.1515/cclm.1982.20.5.299

Trivedi RC, Rebar L, Berta E and Stong L (1978) New enzymatic method for serum uric acid at 500 nm. *Clin Chem* **24:** 1908-1911. DOI: 10.1093/clinchem/24.11.1908

Uchiyama S and Sakamoto H (1997) Immobilization of uricase to gas diffusion carbon felt by electropolymerization of aniline and its application as an enzyme reactor for uric acid sensor. *Talanta* **44**: 1435-1439. DOI: 10.1016/S0039-9140(97)00023-4

Verma S, Choudhary J, Singh KP, Chandra P and Singh SP (2019) Uricase grafted nanoconducting matrix based electrochemical biosensor for ultrafast uric acid detection in human serum samples. *Intl JBiol Macromol* **130**: 333-341. DOI: 10.1016/j.ijbiomac.2019.02.121

Wang Q, Wen X and Kong J (2020) Recent progress on uric acid detection: A review. *Critical Reviews in Anal Chem* **50:** 359-375. DOI: 10.1080/10408347.2019.1637711

Wang X, Yin F and Tu Y (2010) A Uric Acid Biosensor Based on Langmuir-Blodgett Film asan Enzyme-Immobilizing matrix. *Anal Lett* **43**:1507-1515. DOI: 10.1080/00032710903502074

Wang J, Yang S, Cai R, Lin Z and Liu Z (2005) A new method for determination of uric acid by the lactic acid-acetone-BrO(3)(-)-Mn(2+)-H_2SO_4 oscillating reaction using the analyte pulse perturbation technique. *Talanta* **65**: 799-805. DOI: 10.1016/j.talanta.2004.08.008

Wang X, Hagiwara T and Uchiyama S (2007) Immobilization of uricase within polystyrene using polymaleimidostyrene as a stabilizerand its application to uric acid sensor. *Anal Chim Acta* **587**: 41-46. DOI: 10.1016/j.aca.2007.01.025

Xu D-K, Hua L, Li Z-M Chen JH-Y (1997) Identification and quantitative determination of uric acid in human urine and plasma by capillary electrophoresis with amperometric detection. *J Chromatogr B* **694:** 461–466. DOI: 10.1016/S0378-4347(97)00141-2

Yao D, Vlessidis AG and Evmiridis NP (2003) A potentiometric indirect uric acid sensor based on ZnO nanoflakes and immobilized uricase. *Anal Chim Acta* **478**: 23–30. DOI: 10.1016/S0003-2670(02)01484-8

Zanon NCM, Oliveira Jr ON and Kaseli L (2012) Immobilization of uricase enzyme in Langmur-Blodge films of fatty acids:Possible use as a uric acid sensor. *J Colloid Interface Sci* **373:** 69-74. DOI: 10.1016/j.jcis.2011.07.095

Zen JM, Lai YY, Yang HH, Senthil KA (2002) Multianalyte sensor for the simultaneous determination of hypoxanthine, xanthine and uric acid based on perionized nontronite coated screen printed electrode. *Sens Actuat B: Chem* **84**: 237-244. DOI: 10.1016/S0925-4005(02)00031-X

Zhang W, Zhao X and Diao L (2021) Highly sensitive uric acid detection based on grapheme chemoresistor and magnetic beads. *Biosensors* **11**(9): 304. DOI: 10.3390/bios11090304

Zhang F, Li C, Li X, Wang X, Wan Q and Xian Y (2006) ZnS quantum dots derived a reagentless uric acid biosensor. *Talanta* **68**: 1353-1358. DOI: 10.1016/j.talanta.2005.07.051

Zhang F, Wang X, Shiyun AI, Sun Z, Wan Q, Zlu Z, Xian Y, Jin L (2004) Immobilization of uricase on ZnO nanorodes for a reagentless uric acid sensor. *Anal Chim Acta* **519**: 155-160. DOI: 10.1016/j.aca.2004.05.070

Zhang FF, Wang XL, Li CX, Li XH, Wan Q and Xian YZ (2005) Assay for uric acid level in rat striatum by a reagentless biosensor based on functionalized multi-wall carbon nanotubes with tin oxide. *Anal Bioanal Chem* **382**: 1368-1373. DOI: 10.1007/s00216-005-3290-5

Zhang Y, Wen G, Zhou Y, Shuang S, Dong C and Choi MM (2007) Development and analytical application of an uric acid biosensor using an uricase-immobilized eggshell membrane. *Biosens Bioelectron* **22**: 1791-1797. DOI: 10.1016/j.bios.2006.08.038

Zhang YQ, Shen WD, Gu RA, Zhu J and Xue RY(1998) Amperometric biosensor for uric acid based on uricase immobilized silk fibrion membrane. *Anal Chim Acta* **369**: 123-128. DOI: 10.1016/S0003-2670(98)00236-0

Zhao C, Thuo MM and Liu X (2013) A microfluidic paper-based electrochemical biosensor array for multiplexed detection

of metabolic biomarkers. *Sci Technol Adv Matrl* **14**: 054402. DOI: 10.1088/1468-6996/14/5/054402

Zhao FY, Wang ZH, Wang H, Zhao R, Ding MY (2011) Determination of uric acid in human urine by ion chromatography with conductivity detector. *Chin Chem Lett* **22:** 342–345. DOI: 10.1016/j.cclet.2010.10.008

Zhao S, Wang J, Ye F and Liu Y-M (2008) Determination of uric acid in human urine and serum by capillary electrophoresis with chemiluminescence detection. *Anal Biochem* **378:**127–131. DOI: 10.1016/j.ab.2008.04.014

Zhao, Y, Wei X, Peng N, Wang, J and Jiang Z (2017) Study of ZnS nanostructures based electrochemical and photoelectrochemical biosensors for uric acid detection. *Sensors* **17**: 1235. DOI: 10.3390/s17061235

Zinellu A, Carru C, Sotgia S and Deiana L (2004) Optimization of ascorbic and uric acid separation in human plasma by free zone capillary electrophoresis ultraviolet detection. *Anal Biochem* **330:** 298-305. DOI: 10.1016/j.ab.2004.04.009

Zou HL, Li BL, Luo HQ and Li NB (2015) A novel electrochemical biosensor based on hemin functionalized graphene oxide sheets for simultaneous determination of ascorbic acid, dopamine and uric acid. *Sens Actuat B: Chem* **207**: 535-541. DOI: 10.1016/j.snb.2014.10.121