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ESTIMATION A COMPREHENSIVE REVIEW OF CREATININE METHODS: BENEFITS AND PITFALLS

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Abstract:- A crucial biomarker for assessing kidney and muscle health is creatinine, a metabolic byproduct of creatine. For the diagnosis and treatment of renal disorders, especially chronic kidney diseases (CKD), an accurate creatinine measurement is crucial. The fundamental ideas, advantages, disadvantages, and clinical significance of several methods for determining creatinine levels are covered in this article. Despite being susceptible to interference from chemicals like proteins and glucose, the classic Jaffe method—which depends on the interaction between creatinine and picric acid—remains popular because it is inexpensive and simple to use. Although they are more expensive, enzymatic assays, which use creatininase and related enzymes, offer improved specificity and accuracy. For measuring creatinine, sophisticated techniques such as High-Performance Liquid Chromatography (HPLC), Ion-Selective Electrodes (ISE), and Molecularly Imprinted Polymers (MIPs) provide improved sensitivity and dependability. The review also emphasizes how new biosensor technologies are being developed to increase diagnostic accuracy and enable faster and more convenient testing choices. This comprehensive analysis of the different approaches highlights the need for accurate and affordable creatinine measuring methods in clinical and research settings, especially for the early identification and efficient treatment of renal disorders.

Keywords: Creatinine estimation, Chronic Kidney disease,Jaffe method, Enzymatic assay, Biosensors, Molecularly Imprinted polymer (MIP).

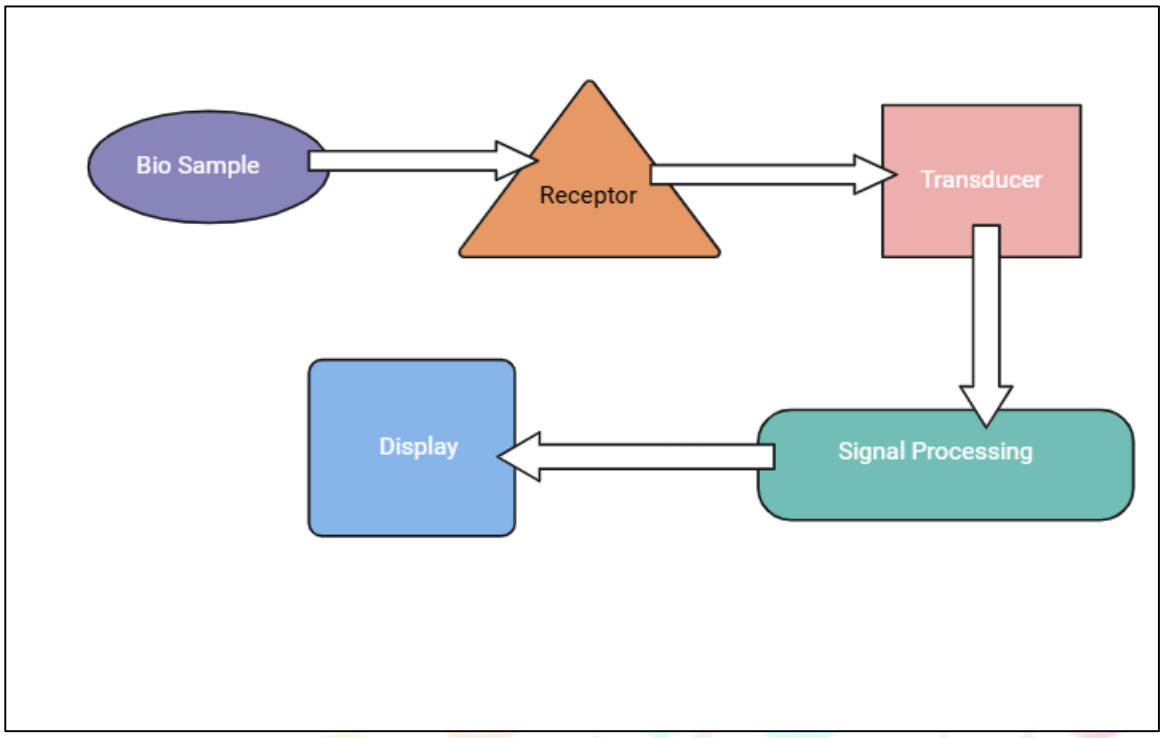
INTRODUCTION:-The kidneys eliminate creatinine, a waste product of the breakdown of creatine, which mostly occurs in muscle tissue. It is produced by enzymatic processes from amino acids including methionine, arginine, and glycine; a little percentage of these amino acids will naturally transform into creatinine. The most crucial markers for assessing renal health, muscle health, and overall metabolic status are measurements of creatinine in the blood and urine[1].Therefore, a high creatinine level could be a sign of a potentially fatal illness like acute renal failure [2].Chronic kidney diseases (CKDs) are amongst the most common conditions that degenerate health throughout the globe, resulting in considerable morbidity and mortality which incurs a massive economic cost to healthcare industry [3] [4] [5]. It is believed that between 11 and 13 percent of people worldwide have CKDs. Kidneys: Filtering systemic blood waste, particularly nitrogenous-based wastes, is the kidneys' main job [6]. The body accumulates the harmful substances (urea and creatinine) based on the patients' and stages of renal failure.

The maximum increases in serum uremic solutes small of molecular weight (expressed as urea, a reference molecule) and intermediate size molecules [creatintine] derived from protein metabolism are a clinically relevant marker for defining kidney dysfunction. Serum urea and creatinine can rise 10 times the normal value in both acute and chronic renal failure conditions [3] [7] [8]. Creatinine (IUPAC name: 2-amino-1-methyl-imidazolium) is a waste product of creatine metabolism in mammals, which takes place in skeletal muscles, where energy is by the way produced! It enters the circulation and is expelled from the body premises at a relatively steady rate by renal process of filtration [9]. Measuring creatinine concentrations has been used to diagnose kidney, thyroid, and muscle issues because these conditions raise the amount of creatinine in blood serum [5]. Specifically, the development of inexpensive, highly sensitive, and selective biosensors for the measurement of creatinine has advanced significantly in recent years. Creatinine (molar mass 113.12 g/mol) concentrations increase from 40–150 μM . Therefore, estimating serum and urine creatinine with urea concentration utilizing easy-to-use, quick, affordable, and reliable methodologies enhances early detection approaches and results in appropriate treatment of patients with renal disease [10].

The biosensors described in this review article are used to quantify creatinine in serum, plasma, urine, and blood samples. Creatinine measurement in a variety of biological matrices is made easier by the biosensors covered here. Molecular Imprinted Polymers (MIPs), cells, enzymes, antibodies, and biological tissues are examples of common receptor types. The transducer keeps an eye on how the bio-analyte and receptor interact. The information regarding the different techniques used to estimate creatinine was mentioned in this paper. Using Jaffe's approach, the concentration of creatinine is directly proportional to the color intensity of the orange-red complex that is created when it reacts with alkaline picrate. Its most popular approach. For measuring creatinine, the enzymatic approach is advised because it is far more accurate and has just recently been made accessible for clinical usage. both picric acid-based Jaffe assays and enzymatic tests. The Jaffe assays are more susceptible to interfering substances in terms of both frequency and degree of interference, even though enzymatic assays are not immune to non-specificity. Some authors advocate using the enzymatic assay rather than the Jaffe assay. . Despite being less prone to non-specificity bias, the enzymatic assay is substantially more expensive than the Jaffe assay. Other methods are HPLC, MIP, ION Selective Electrode. The ion-selective electrode (ISE) method for creatinine estimation is a potent analytical approach that exploits the selective response of electrodes to certain ions, permitting reliable determination of creatinine levels in biological samples. This approach is particularly advantageous due to its simplicity, rapid reaction, and low interference from other chemicals. For the determination of creatinine, High-Performance Liquid Chromatography (HPLC) has a number of benefits, especially with regard to efficiency, accuracy, and precision. Because it requires little sample preparation and yields dependable findings, this approach is becoming more and more popular in clinical and pharmaceutical contexts. The MIP (Molecularly Imprinted Polymer) method is a promising analytical technique that improves the sensitivity and specificity of biochemical assays for creatinine quantification. This technique uses the special qualities of molecularly imprinted polymers to bind creatinine selectively, enabling precise measurement in a range of biological materials. [11] [12].

2.Receptors of creatinine biosensors:-

- Enzyme
- MIP
- Antibody

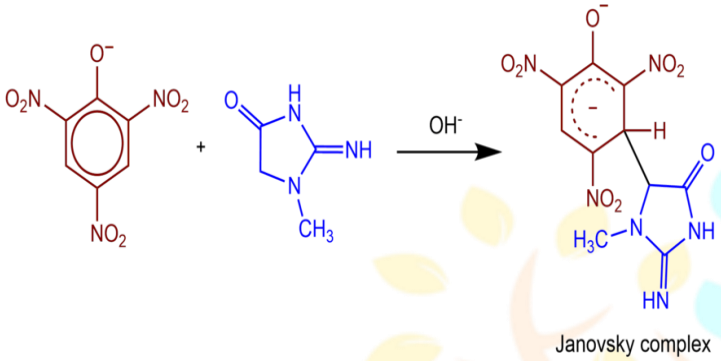


are all used in the creation of MIPs. To create a coherent polymer chain, crosslinkers **2.1 Enzymes:-** 2.1 Creatinine is converted by enzymes into other measurable compounds (NH4 or H2 O2). Transducers for NH4 (a pH detection electrode) and H2O2 (an oxygen detection electrode) were developed as a result of earlier developments, and the concentrations of these transducers were directly correlated with serum creatinine levels. The enzymatic technique provides an illustration of the particular enzymes used in creatinine sensors. Although the detection of creatinine using sequential enzymes exhibits a high degree of selectivity, this technology has significant expensive implications. The activity of the enzyme itself limits how long an enzyme-based sensor can operate. In laboratory settings, both enzyme-based and Jaffe-based sensors are widely used. To act as receptors, enzymes can be combined with optical, electrochemical, and ISFET-based transducers. As mentioned before, the immobilization technique can have a big impact on how long the sensor lasts. [14]

2.2 Molecularly imprinted polymers (MIPs): Molecularly imprinted polymers (MIPs) act as synthetic bioanalyte receptors. They are known to be useful instruments for imitating receptor recognition sites. Monomers, crosslinkers, and template molecules join monomers. In biological samples, templates with unique shapes are extracted from the polymer, creating corresponding spaces for target molecules. By avoiding interference from other substances, the proper creation of these cavities is ensured by the careful selection of crosslinkers. In addition to molecular imprinting, the "Bite-and-Switch" technique is used to improve material selection for creatine and creatinine detection. This technique imprints polymers with methylated template analogs to improve crosslinking in MIPs. Grafting photo-polymerization yields highly crosslinked MIPs with exceptional stability and sensitivity. The partial elimination of templates is a significant drawback of MIPs. Significant stability, sensitivity, and selectivity are among MIPs' advantages. With quicker synthesis times than other sensors, they demonstrate exceptional physical and chemical resistance to a variety of degrading agents, solvents, metal ions, and acidic environments [13]. MIPs for creatinine detection have been developed using a variety of monomers and crosslinkers. Examples include polyethylene-co-vinyl-alcohol (EVAL), 4-vinylpyridine (4-Vpy) combined with divinylbenzene as a crosslinker, and 2-acrylamido-2-methyl-1-propanesulfonic acid (AMPS) as a monomer and N,N-methylenediacrylamide (MBA) as a crosslinker. Other combinations include of β-cyclodextrin (β-CD) as monomers with epichlorohydrin (EPI) as crosslinkers, and melamine (mel) and chloranil (chl) with a low-level crosslinking network. Additionally, ethylene glycol diglycidyl ether is used to crosslink polymethacrylic acid. MIPs can be used as receptors in electrochemical, capacitive, ISFET-based, and chromatographic sensors [13] [14].

Antibody:- (as a receptor) is employed in amperometric sensors to quantify creatinine. The section on amperometric sensors will cover the detection and measuring mechanism.

JAFFES RESPONSE SYSTEM: The Jaffe response system's basic idea One commonly used laboratory technique for measuring serum creatinine levels is the Jaffe response system. Chemical reaction The reaction of alkaline picric acid with serum creatinine is known as the Jaffe response. The reddish complex produced by this reaction is used as the foundation for measuring creatinine levels. Absorbance Dimension The intensity of color produced during the reaction is measured at a certain wavelength, usually between 490 and 510 nm. The amount of creatinine present in the serum sample is closely correlated with the absorbance of this colored complex [15].



Janovsky complex

The orange-red creatinine picrate complex is made up of creatinine plus picric acid. Serum creatinine was measured in the biochemistry department using an Abbot Architect ci 8200 auto analyzer and the Jaffe method. When creatinine and picric acid mix in an alkaline solution, the result is an orange creatinine picrate hue at 520 nm [16] Jaffe's method, which was first published in 1886 and is based on the alkaline picrate method with some modifications to remove interference, is the most popular methodology for creatinine quantification. The reaction was used to measure creatinine levels by Folin and Wu (1919) [17] Jaffe's reaction has been used to assess creatinine in serum and urine samples. Creatinine is transported by the bloodstream to the kidneys for excretion as a consequence of creatine metabolism [18].

Different Approaches There are three main variations of the Jaffe response. The Kinetic Jaffe Technique: This approach minimizes interference from quickly reacting compounds like keto-acids by monitoring the synthesis of the picrate-creatinine complex over a specified time period. In order to obtain more accurate creatinine readings, measurements are made over a period of up to 120 seconds [19].The Fixed Time Jaffe Method With this method, the color intensity is measured at a specific point in time after the reaction has begun. The concentration of creatinine directly correlates with the color's intensity [20].

The End-Point Jaffe Method: This method uses a two-step procedure to create the initial orange-red complex and then decolorize it by adding an acidic reagent. Although this approach is more vulnerable to interference from non-specific chemicals, the degree of decolorization is correlated with the creatinine concentration [19]. Interferences: The lack of specificity of the Jaffe reaction as a result of interference by molecules like proteins, ascorbic acid, and keto-acids is a severe drawback [20] [21]. Inaccurate creatinine readings could arise from this influence [22].

Remedial Actions Several corrective measures, such as pre-incubation with sodium hydroxide and treatment with trichloroacetic acid or potassium ferricyanide, can be used to improve the specificity of the Jaffe reaction [19].

1. Pre- incubation with sodium hydroxide: Sodium hydroxide (NaOH) reduces the damage that bilirubin causes by functioning as an oxidizing agent and converting bilirubin into biliverdin. [23] [24] [25] [26].

2. Trichloroacetic acid treatment: The impact of trichloroacetic acid on bilirubin interference indicates that there is a significant difference between the serum creatinine readings with high bilirubin before and after the

addition of trichloroacetic acid. One of the best methods for reducing serum creatinine levels of all bilirubin kinds is this technique. There is a direct correlation between bilirubin attention and bilirubin hindrance [27].

3. Potassium ferricyanide treatment: Bilirubin impediment is prevented by potassium ferricyanide, an oxidant, which oxidizes bilirubin to biliverdin prior to the alkaline picrate result being added to the serum sample [28].

4. Addition of sodium dodecyl sulphate: Sodium dodecyl sulphate (SDS), an alkaline buffer, promotes bilirubin's release from albumin and aids in its complete oxidation to biliverdin [23].

5. Addition of Fullers Earth(FulE): The inclusion of FulE is a less well-known method of preventing bilirubin impediment. If there is no interference, FulE completely adsorbs the bilirubin [19].

APPLICATIONS: A well-known technique for measuring creatinine levels in serum, especially in clinical settings, is Jaffe's method. The following highlights important elements related to its application: Historical Context: The process developed by Jaffe, first described in 1886, is based on the reaction of creatinine with picric acid in an alkaline solution, which produces a colored complex that can be measured quantitatively at 500 nm by spectrophotometry. Numerous changes have been made to this method in an effort to reduce interference from extraneous compounds found in serum. [29] Common Use: In many clinical laboratories, Jaffe's method is still the most often used technique for creatinine measurement, despite the fact that there are other approaches available. Its cost-effectiveness in comparison to enzymatic methods, which are linked to larger financial expenditures, makes it especially preferred [30]. Jaffe's method is commonly compared to enzymatic procedures and the industry standard isotope dilution mass spectrometry (IDMS) in clinical use. Despite being widely used, empirical research has shown that Jaffe's method can sometimes yield results that differ from those obtained using enzymatic techniques. One study highlighted a mean difference of 0.0685 mg/dL between the enzymatic and Jaffe's techniques, for instance, indicating a respectable level of correlation overall [31].

Point of Care Testing (POCT): For quick estimates of creatinine levels in emergency medical situations, point of care testing equipment usually uses enzymatic procedures. However, when these devices' results diverge from Jaffe's approach, it could lead to misunderstandings and potential poor patient care management. As a result, Jaffe's approach serves as a reliable guide for validating creatinine levels when disparities are noticed [32]. Statistical Validation: High intraclass correlation coefficients (ICCs) reported in studies, which indicate strong agreement with other approaches, support the reliability of Jaffe's approach. An ICC of 0.995, for example, has been documented, suggesting a remarkable correlation between enzymatic and Jaffe's methods [33].

LIMITATIONS:-1.Bilirubin Interference: The study shows that bilirubin can significantly interfere with creatinine measurements using the Jaffe Kinetic technique. Specifically, bilirubin levels higher than 20 mg/dl might lead to erroneous "out values" when creatinine is measured without first incubating with NaOH. This implies that the approach might not be reliable for patients with high bilirubin levels, which are commonly seen in cases of liver disease [15].

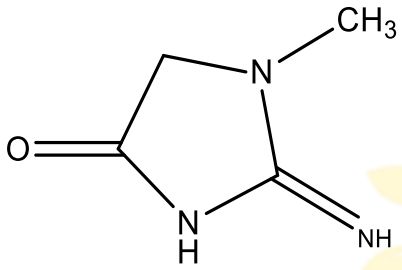
2. Interference by Other compounds: Jaffe's method is known to be susceptible to interference from a number of compounds present in serum, such as bilirubin and glucose. This vulnerability may lead to imprecise measurements of creatinine levels, which is a significant drawback in clinical settings where accuracy of findings is crucial [16].

3.Cost Implications: Although Jaffe's approach is widely used since it is less expensive than enzymatic methods, this cost-effectiveness may occasionally come at the expense of precision. Since enzymatic methods are typically less impacted by nonspecific biases, clinical laboratories might prioritize cost reductions over the improved accuracy they can provide [16].

4.Chromogenic Interferents: The analytical process under consideration exhibits a significant vulnerability to interference from a variety of chromogenic agents, including uric acid, glucose, and particular antibiotic classes. Both positive and negative interference may be caused by these substances, which could lead to an incorrect creatinine level measurement [28].

5. Poor Agreement with Other Measurement Techniques: Empirical studies have shown that the Jaffe method often lacks concordance with other creatinine measurement techniques, particularly in samples that have icterus. Significant differences in creatinine concentrations reported by different techniques may result from this divergence [28].

ESTIMATION OF CREATININE BY ENZYMATIC METHOD:



Creatinine

INTRODUCTION: The most requested measure in renal function testing at the moment is creatinine, which is essential for clinical follow-up and medical care, particularly for kidney transplant recipients. There are several methods for measuring this parameter. The MDRD equation and all other formulas for predicting glomerular filtration rate (GFR) are impacted by the present variability in serum creatinine readings. Accurate and precise measurements are necessary for SKF's more reliable evaluation in order to establish a trustworthy clinical judgment. The propelled technique and the enzyme method are the two primary method types that are extensively described in the literature. According to some research, the Jaffe method of assessing serum creatinine has significantly more variability than enzymatic methods when compared to reference values.

• The fundamental idea behind enzymatic approaches is a sequence of enzymatic processes that produce a colored end product, the degree of which is precisely proportionate to the creatinine content.

• The most widely used enzymatic method uses the enzyme "creatininase," which converts creatinine to creatine, which is then converted to sarcosine by creatininase. A final enzymatic reaction that varies depending on the source measures the amount of formaldehyde, glycine, and hydrogen peroxide produced after sarcosine is transformed by the enzyme sarcosine peroxidase [34].

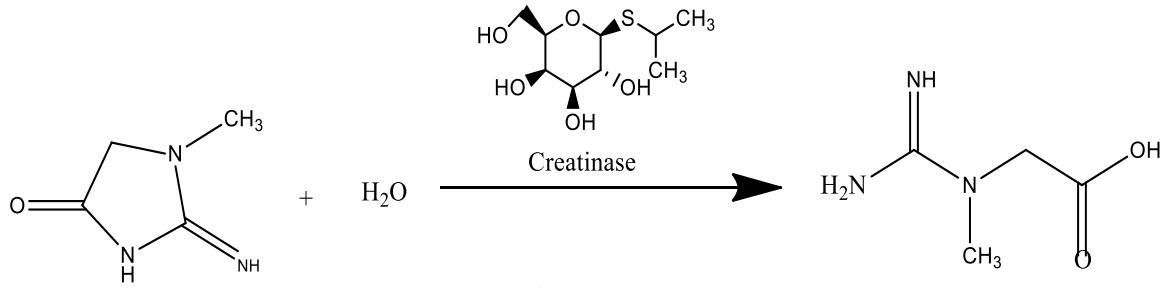
• The enzymatic method, which is much more accurate and has only been available for clinical use, is recommended for creatinine quantification [35].

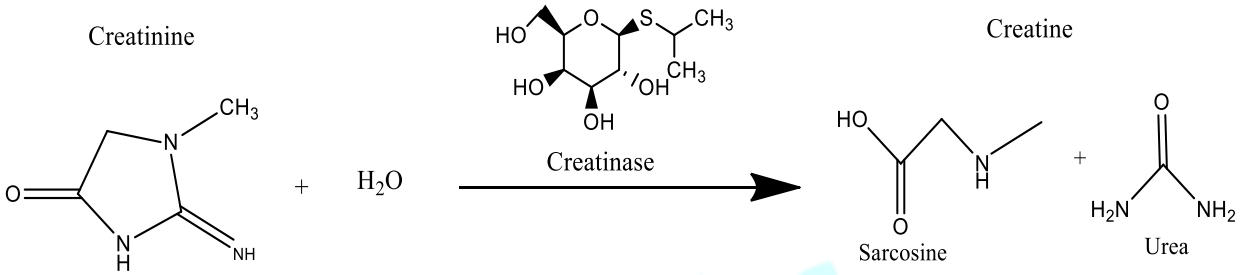
• Creatinine is also estimated by point-of-care testing equipment since therapy requires faster test results. To determine the creatinine content of whole blood, the Radiometer ABL800 FLEX blood gas analyzer uses an amperometric biosensor (electrode) based on the enzymatic conversion of creatinine to sarcosine with the generation of hydrogen peroxide. Despite being somewhat more expensive than the Jaffe assay, the enzymatic assay is less likely to display nonspecific bias. The majority of labs utilize the Jaffe method for creatinine measurement to cut test costs. In emergency cases, creatinine is estimated using the enzymatic technique of the POCT device in a number of tertiary institutions, and treatment decisions are based on the results. When the POCT device's results are questionable [36].

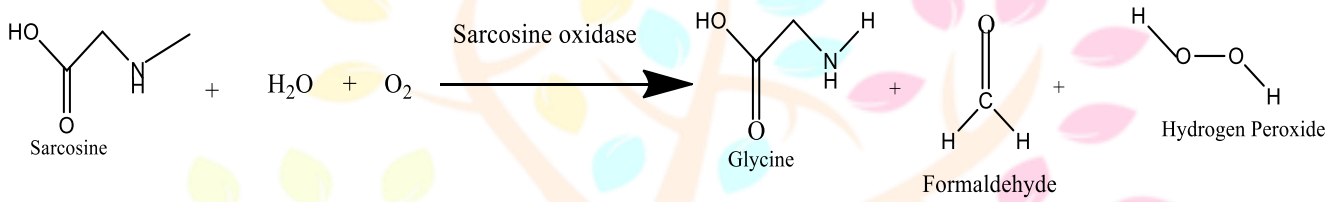
• Specialized protein structures called enzymes play a vital role in catalysis. They don't influence the equilibrium of chemical reactions and stay the same throughout them. Similar to locks and keys, enzymes

only interact with specific substrates and have an impact on specific molecules. Here, creatinine acts as a substrate, and enzymes' job is to convert it into components that can be measured without influencing other substances. Furthermore, the active site is a location where enzymes and the substrate are revived together. The use of enzymes in bioassays is restricted by a number of criteria, including substrate concentration, ionic strength, enzyme lifespan (activity), environmental conditions (including pH and temperature), and the high costs of their synthesis and purification. Techniques for immobilization and structural alterations can help overcome some of these restrictions. The following enzymes are used in creatinine sensors to convert creatinine into compounds that may be measured.

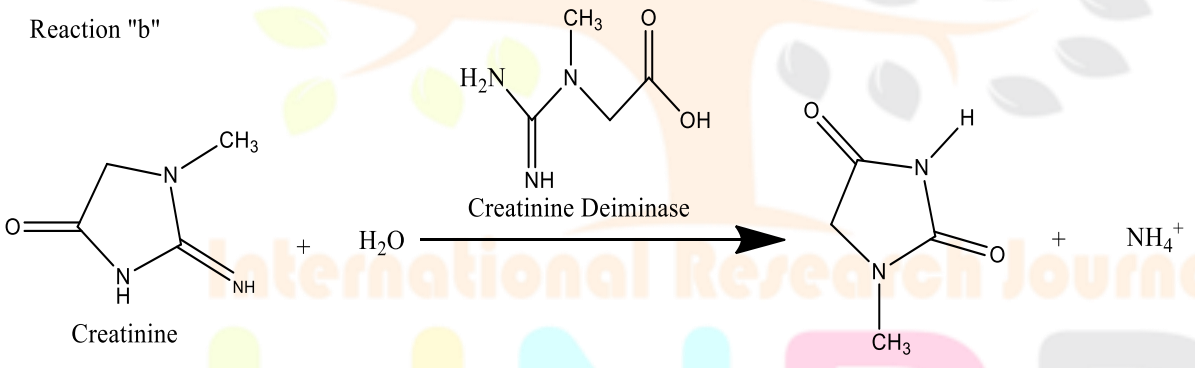
Enzymatic reaction "a"



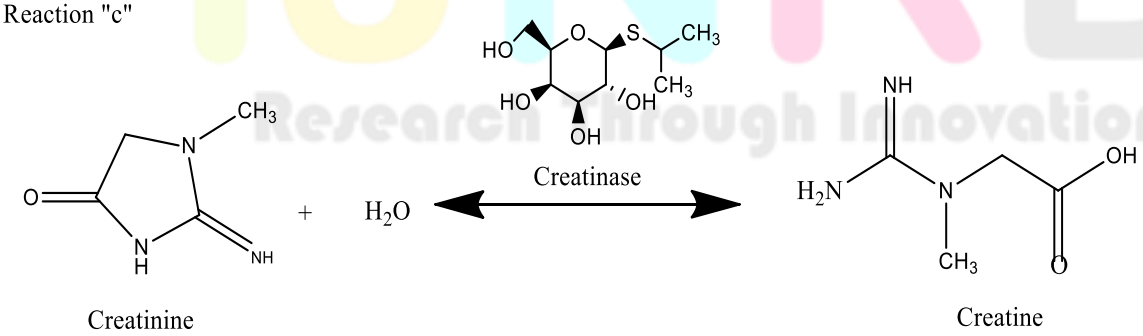




Reaction "b"



Reaction "c"



PRINCIPAL:-

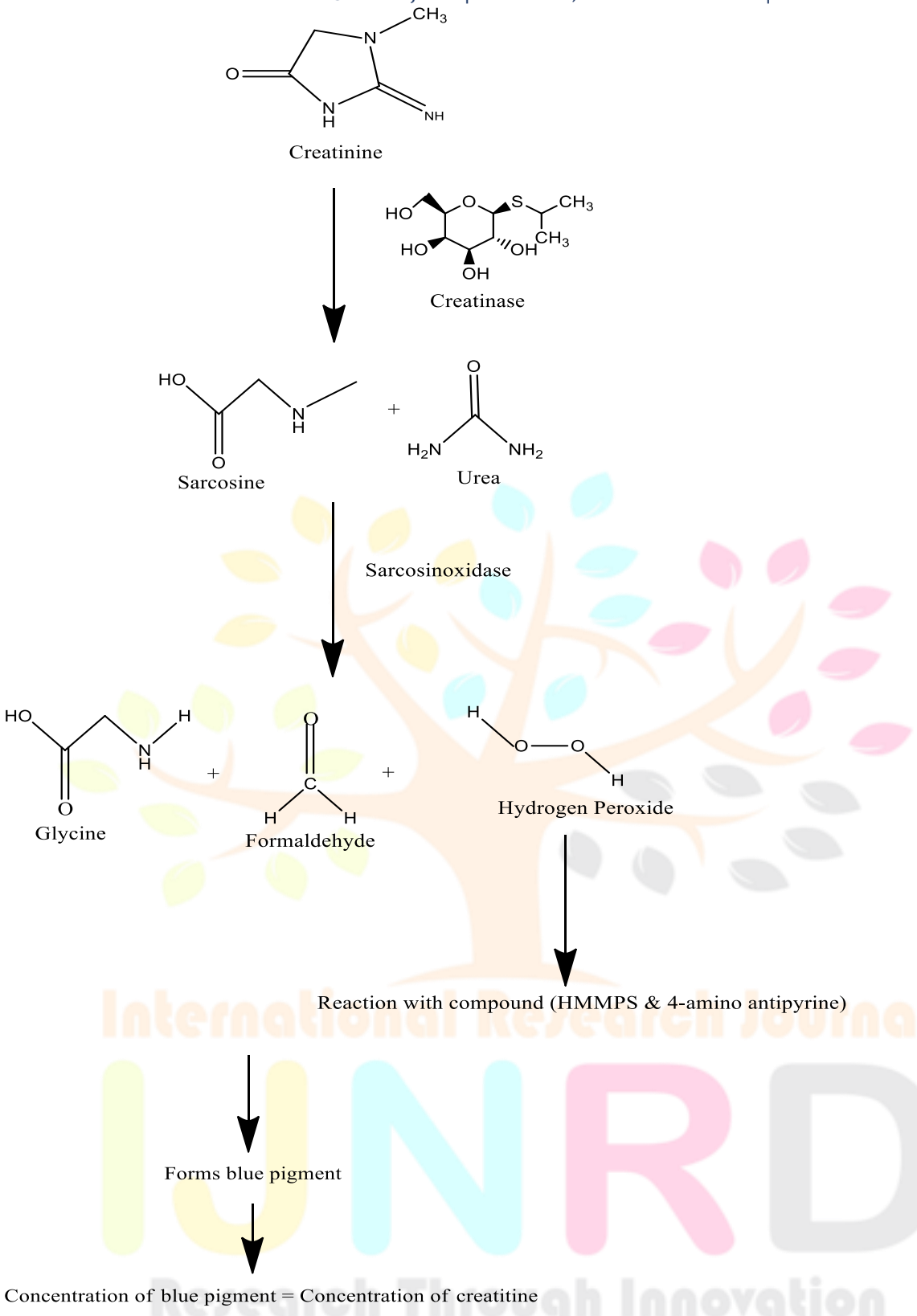
Enzymatic Reaction Method:-

1. **Enzyme Activation**:The test makes use of enzymes, which are unique proteins that accelerate chemical reactions. The process for creatinine is initiated by the enzyme creatininase.

2. **Reaction Steps**: The material we wish to measure, creatinine, is broken down into creatine by creatininase. Next, creatine is converted to sarcosine by an additional enzyme known as creatinase. Sarcosine is broken down by the enzyme sarcosine oxidase, releasing chemicals such as hydrogen peroxide.

3. **Color Change for Measurement**: Hydrogen peroxide reacts with other chemicals to create a colorful material in the end. The amount of creatinine in the sample is indicated by the color's intensity.
4. **Measuring the Color**: The color intensity is measured by a machine. The more creatinine there is in the sample, the deeper the colour Because each reaction is very specific to creatinine, this enzymatic approach is favored for its precision over alternative techniques that may result in unintended side effects.

   • Reaction "a" uses the H2O2 generated during the three enzyme process to allow amperometric biosensors to measure creatinine levels. Electrodes from oxygen detectors also contribute to this response. On the other hand, reaction "b" uses the NH4 produced as a transducer to measure creatinine levels using instruments like pH sensors and potentiometric biosensors. One of the main benefits of this enzyme-only process is how simple it is. The creation of potentiometric creatinine biosensors uses reaction "c" in a similar way. . The design and execution of these sensors have been complicated by the complex nature of the three-enzyme systems, a reduction in sensitivity ascribed to the enzymes' presence, and dual systems that differentiate between creatine and creatinine. Enzyme immobilization is the method by which an enzyme is joined to an inert substrate to create a unique solid matrix [37].
5. In the enzymatic analysis, creatinease hydrolyzes creatinine to create urea and sarcosine. Sarcosioxidase catalyzes the oxidative demethylation of sarcosine, which results in the formation of glycine, formaldehyde, and hydrogen peroxide. The medium's hydrogen peroxide reacts with 4-aminoantipyrine and N- (3-sulfopropyl)-3-methoxy-5-methylaniline (HMMPS) to produce a blue pigment when peroxidase (POD) is present. The concentration of creatinine is correlated with the change in absorbance at 600/700 nm [38].



Applications: 1. Less Interference: It is well known that enzymatic techniques are less susceptible to interference from chemicals that could compromise the precision of the results. This increases their dependability, particularly in complex biological samples such as kidney transplant recipients' samples.

2. Analytical Performance: These techniques have outstanding analytical performance, which is essential for kidney function monitoring. Precise measurements are made possible by the enzymatic processes that produce a colored product whose intensity precisely correlates with creatinine content.

3. Specificity and Standardization: The reliability of enzymatic methods is increased by their specificity and standardization. They are not as susceptible to changes brought on by pseudochromogenic chemicals, which is a typical problem with colorimetric techniques like the Jaffé method.

4. Correlation with Reference Values: Research shows that when compared to reference values, enzymatic methods exhibit significantly less variability than Jaffé procedures. Since precise creatinine levels are necessary for medication modifications, this constancy is critical for the efficient medical management of kidney transplant patients.

5. Cost Considerations: Although enzymatic techniques have many benefits, their higher cost may prevent many laboratories from using them widely. When selecting testing procedures, healthcare facilities need to take this important element into account.

6.Clinical Relevance: As it helps with the continuous evaluation of kidney function and the modification of immunosuppressive treatments, the capacity to produce accurate and dependable serum creatinine readings is especially crucial for kidney transplant recipients.

7. Enzymes are protein structures that have a special catalytic function. They are not consumed in a chemical reaction, nor do they change its equilibrium.

8. Because of their better analytical capabilities and lower interference, they come highly recommended.

9. Is made up of a sequence of enzymatic processes that result in a colorful end product, the intensity of which is directly related to the level of creatinine.

10. According to the literature, colorimetric techniques are vulnerable to interference from pseudochromogenic chemicals, while enzymatic techniques are standardized due to their performance and specificity.

11. Enzymatic creatinine measurement was created in order to reduce bias, improve accuracy, and get around these interferences.

12. It is much more accurate and recommended for creatinine measurement.

13. The colorimetric method is a simple, reasonably priced.

Limitations: 1. Higher Cost: The cost of enzymatic procedures is typically higher than that of colorimetric methods, which may prevent many laboratories from using them extensively. This expense element may be a major deterrent, particularly in environments with limited resources.
2. Complexity of Procedures: Compared to the simple colorimetric approach, the enzymatic method may include a sequence of enzyme processes that are more complicated. Because of this intricacy, laboratory staff may need more specific training to guarantee reliable results.
3. Specificity Problems: Although enzymatic techniques are renowned for their specificity, some compounds in the serum may still have an impact on them and obstruct the enzymatic reactions. Results may vary as a result, though typically not as much as with colorimetric techniques.
4. Dependency on Reagent Quality: The stability and quality of the reagents used can have a significant impact on how well enzymatic procedures work. The precision and dependability of the results can be impacted by variations in reagent quality.
5. Limited Availability: The use of enzymatic methods in everyday practice may be restricted since not all laboratories have access to the required tools or reagents.
6. Potential for Interference: The presence of specific medications or metabolites may nevertheless have an impact on the precision of creatinine readings, even when hemolysis does not substantially impede enzymatic procedures.
7. Environmental variables (pH and temperature) and enzyme concentration have an impact on the outcomes.
8. Enzyme production and purification are extremely expensive.
9. The primary problems with the colorimetric approach are the disturbing color shift in the sample and the interference of other materials in the chemical process.

10. However, their exorbitant cost prevents the majority of laboratories from using them.
11. Their low utilization and high cost in comparison to colorimetric methods have probably limited their application in analytical laboratories.

12. It has been discovered that enzymatic creatinine measurement is considerably more expensive and less prone to interferences than Jaffe.
13. In addition to their advantages, these point-of-care testing (POCT) devices have a number of drawbacks.

High performance liquid chromatography:

Chromatography: One of the earliest and most widely used techniques for separating large amounts of constituent materials is chromatography. After the analyte has been extracted or separated, analyze it with a spectrophotometer or other detection tool. HPLC, which allows us to identify, separate, and measure a variety of compounds, is one of the most important tools in chromatography for analysis. To meet that HPLC requirement, we moved and displaced our components into a high pressure mobile liquid phase column [39].

CREATININE DETERMINATION BY HPLC WITH UV DETECTION

Principle: The most sensitive HPLC technique for measuring creatinine is reversed-phase transfer chromatography with pre-column derivatization, which completely isolates the substance to be measured from the interference. The creatinine peak is then tracked by total absorbance at 236 nm using a UV detector. This approach can be performed with either serum or urine samples and yields good overall agreement when compared to data collected using other methods [40] [41].

Material: Urine samples Serum or plasma samples 1. Octanesulfonic acid (10 mmol/liter; 2.2 g/liter), sodium salt, 95:5 water/acetonitrile Orthophosphoric acid Creatinine standards, which range from 0 to 200 µmol/liter Drugstores Pump Model 2248 of LKB Kratos Analytical Model Spectroflow 783 detector (other HPLC/detector systems can be utilized, although results may vary). column for analysis (Macherey-Nagel) Nucleosil 120 × 3 mm C18 [40].

Regarding urine specimens Preparing the sample:-

a.Centrifuge 800 × g of urine for five minutes.
b. 200 µl was injected following the dilution of 10 µl of the supernatant in 990 µl of HPLC mobile phase (see to step 3 below).
For serum or plasma samples
c.After three minutes of incubation, a mixture of 100 µL serum and 100 µL acetonitrile should be centrifuged at 2400 × g for ten minutes at [5°C].

d. Mix 950 µl of mobile phase with 200 ul of diluted supernatant.
This quick and simple process is generally equal to measurements made using the Jaffe principle. It removes contaminants that could distort the creatinine result, which makes it effective with difficult samples, particularly serum and plasma. Sample preparation can be completed in 20 minutes, and each control/sample run takes 10 minutes to complete [41] [42].

CREATININE DETERMINATION: LC-MS/MS METHOD

Principle:

This method is frequently used in our lab to quantify creatinine (together with related compounds creatine and guanidinoacetate) in blood and urine (Young, Struys, & Wood, 2007). After a simple isotope dilution/deproteination procedure, a quick HPLC separation and tandem mass spectrometry detection are performed. When an isotopelabeled creatine internal standard (ISTD) is provided to each sample, the

endogenous and ISTD creatinine peaks are tracked using multiple reaction monitoring (MRM) transitions of m/z 114 > 44 and 117 > 47, respectively. Creatinine levels are calculated by comparing the results to a synthetic calibration curve [43] [44].

Material: d3-Creatinine (CDN Isotopes; ISTD) Quality control (QC; pooled residual samples), creatinine acetonitrile, and ammonium acetate. Alternative HPLC/MS/MS systems may be used, however results may vary. Alliance 2795 (Waters) HPLC model Model Quattro Micro API (Waters) tandem mass (MS/MS) spectrometer. HPLC column and the TSK gel Amide-80 Costar Spin-X (Corning) HPLC microcentrifuge filter from Tosoh Biosciences. [45] This method is reproducible, highly accurate, and sensitive. When observing the appropriate standard and ISTD transitions, there is no obvious cross contamination. Starting sample sizes as small as 1 µl (urine) and 2 µl (serum/plasma) have been used successfully. While sample preparation takes about 30 minutes, the calibration curve can be completed in 60 minutes. Each sample or control will then run for eight minutes, and sample analysis will take 10 minutes. A five-sample run takes about two and a half hours [43].

Isotope dilution mass spectrometry (IDMS):-

Isotope dilution mass spectrometry (IDMS) is the gold standard technique for measuring serum creatinine levels. This technique involves adding a predetermined amount of labeled creatinine to the serum sample and letting the labeled and unlabeled creatinine balance at room temperature. The chemicals are subsequently absorbed by an ion exchange material. The creatinine and internal standard are eluted using an ammonia solution following a water wash. Trimethylsilyl derivatives are then produced via a reaction with the isolated creatinine. During gas chromatography, coupled gas chromatography-mass spectrometry (GC-MS) is used to continually monitor the reaction products. A fused silica capillary column is filled with the m/z values of 332 and 329, which stand for the molecular masses of the labelled and non-labelled analytes, respectively. The isotope ratios in the serum samples and a set of standards with preset mixtures of the labeled and non-labelled analyte are used to compute the analytical results [46] [47].

Limitations: 1. Certain compounds may still interfere with the analysis, even though HPLC is less susceptible to outside interference than colorimetric methods. Careful sample preparation and chromatographic settings are necessary to lessen these interferences [39].
2. HPLC analysis can be more expensive and time-consuming than simpler methods like colorimetric testing [39] [40].
3. Complex Sample Preparation: HPLC requires careful sample preparation, including deproteinization and filtration, to remove interfering compounds [48].
4. Due to their complexity, HPLC systems require specialized skills to operate and maintain [49].

5. Despite its high accuracy and precision, HPLC may not be able to detect very low quantities of creatinine [49].

Application: 1. HPLC, which accurately measures creatinine levels in blood and urine, can be used to diagnose and monitor chronic kidney disease (CKD) and other renal disorders [44].
2. HPLC is used to detect creatinine in animal models to study renal function, drug effects, and the onset of kidney diseases [46].
3. By serving as a reference technique for creatinine quantification, HPLC guarantees the accuracy and precision of common clinical procedures [46].

4. Creatinine levels are a measure of an athlete's muscle mass and metabolic rate. HPLC provides accurate measurements to monitor these parameters and detect potential doping activities [48].

5. HPLC measures creatinine levels in biological fluids to assess the impact of drugs on renal function.

MOLECULAR IMPRINTED POLYMERS (MIPS):

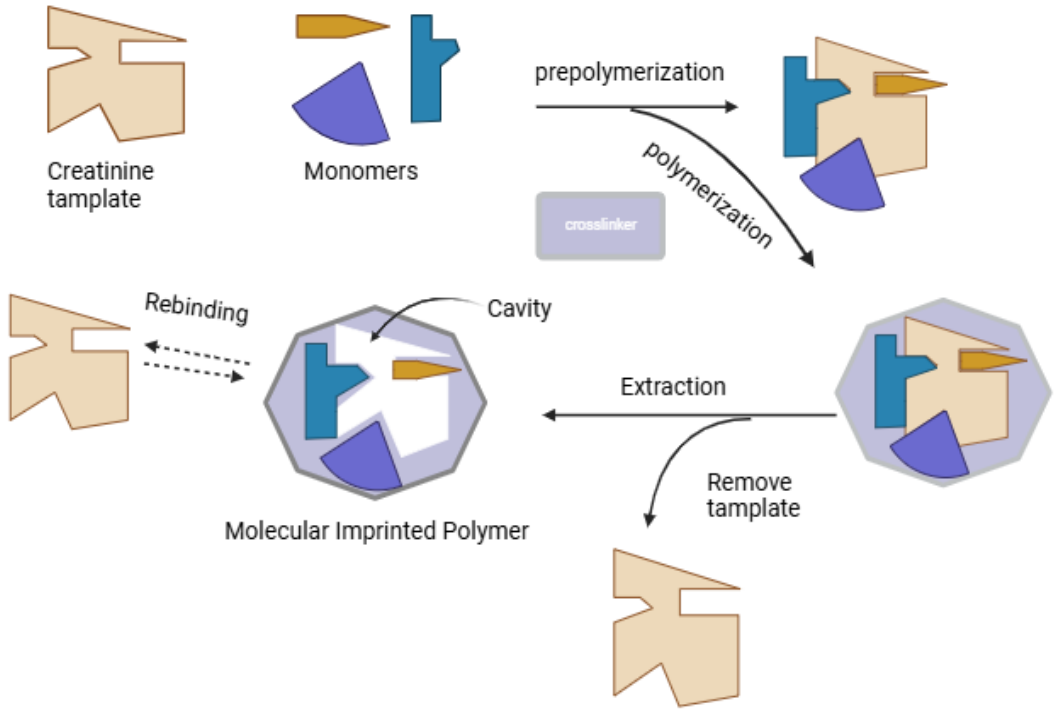
By creating template-shaped voids in polymer matrices, molecular imprinting offers high-affinity selectivity that is predetermined. It acts as an artificial receptor bioanalyst by using a "lock and key" kind of substrate recognition. When coating on substrates and template molecules, the choice of functional monomers is crucial. The template must be removed in order to reveal cavities where target molecules can bind and be measured using various techniques. Impedimetric, capacitive, optical (or other absorption mode), amperometric, and chromatographic techniques are used for estimation [49].

Molecularly Imprinted Polymers (MIPs) Principle:

MIPs can preferentially recognize particular target molecules (like creatinine) thanks to a mesmeric mechanism for molecular recognition and binding. According to this principle: Important facets of this principle include: • Molecule Template: o Here, creatinine serves as the template molecule and is a fundamental step in the process. An OET file of this has been displayed here both at the beginning and following NPT equilibration, respectively. The new molecule is crucial because it identifies the binding sites that will form as well as the polymer matrix that will result from synthesis. used powdered creatine [50] [51].
• Functional Monomers: These are functional monomers that are mixed with the template molecule, such as methacrylic acid (MAA). Because the monomers complement the template through hydrogen bonds and van der Waals forces, among other mechanisms, their complex will be crucial in directing a future "real" polymerization process.
• **Polymerization Process:** The polymerization reaction is started by the addition of a cross linker such as Divinylbenzene (DVB) or EGDMA (ethylene glycol dimethyl acrylate). This results in the template "locking" into position inside the three-dimensional structure of this molecular ink by embedding it in a network of polymers. After sealing the flask, we place it in a water bath that has been prepared to 60°C for a full day of thermal curing.

• **Cavity Formation:** Following polymerization, the template molecule is extracted using soxhlet extraction with refluxed alcohol, resulting in a dry powder with well-defined cavities that are chemically and vent-functionally compatible with a target component. This is the source of MIPs' "molecular memory."
• **Selective Binding**: When the polymer is exposed to a solution containing the target molecule, its cavities specifically identify and bind to creatinine (target:creatinine). Customized interactions between the target molecule and certain functional groups inside these cavities are what give rise to this specificity.

Measurement and Detection:
The quantification of target molecule binding allows for the assessment of target concentration in various samples. For his part, a number of variables, including pH, contact time, and initial target molecule concentration, might influence how effective this process is [52] [53].



Limitation Associated with MIPs

A significant class of materials known as molecular imprinting polymers (MIPs) are designed to specifically recognize and bind target compounds like creatinine. However, they do have a unique set of drawbacks.

Template Removal Issues:

The primary disadvantage is that, once polymerization occurs, it is nearly impossible to remove the template molecule. There is a risk of measuring target molecules incorrectly if the leftover template can obstruct the binding sites [52].

Selectivity Issues: Problems with Selectivity: Although MIPs are meant to be selective, they can react with compounds that share structural similarities. This may result in reduced specificity or false positives when measuring a target analyte, such creatinine, in complete biological samples.

Reproducibility Concerns: Variability of MIP synthesis from batch to batch Results may also vary depending on the raw components and polymerization circumstances. This could cast doubt on a lab's MIP production procedures, depending on the system run.

Limited Binding Capacity: Although the MIP recognition site may be limited, a high binding capacity allows the maximum quantity of target to be bound at greater concentrations. Its usage in high-sensitivity applications, including clinical diagnostics, may be restricted by the latter. Getting a prepared process can be complicated.

Cost and Time : The utilization of particular functional monomers and cross-linkers, as well as usually intricate and solvent-intensive synthesis processes. This adds time to the procedure and may necessitate expensive equipment and reagents.

Stability and Durability: When exposed to various environmental factors, such as changes in pH and temperature, some MIPs may become unstable. Additionally, this may negatively impact their general quality and durability for careers in the field [50] [54] [55].

Conclusion: An key indicator of kidney function, creatinine is a waste product that is innocuous and unnecessary for the body. Different techniques for estimating creatinine each have unique benefits and drawbacks. The accuracy of the Jaffe method can be impacted by interferences from chemicals like glucose

and bilirubin, despite its widespread use due to its affordability and ease of use. Despite being more precise, enzymatic techniques are typically more costly and call for more sophisticated equipment. Although high-performance liquid chromatography (HPLC) has great sensitivity and precision, it usually takes a long time and needs specific equipment. Selecting the best approach based on clinical circumstances is essential since molecular impairment, such as genetic mutations or kidney failure, can affect the accuracy of some creatinine measuring procedures. Every technique has a role in both clinical and scientific contexts. Because of their effectiveness and accessibility, techniques like enzymatic assays and Jaffe are frequently used for everyday clinical applications. For specialized applications, such pharmacokinetics or toxicology, where precision is crucial, HPLC and other sophisticated methods are more appropriate. Future developments in creatinine estimation will probably try to improve accuracy for both ordinary and specialized use cases while overcoming the drawbacks of existing techniques, particularly with regard to interference, cost, and usability.

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