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NOVEL METHOD FOR DIABETES DETECTION: NO PRICK, NO PAIN

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- **ABSTRACT**

Diabetes is a condition that happens when blood sugar Mi(glucose) is too high. It develops when pancreas doesn't make enough insulin or any ata ll, or when body isn't responding to the effects of insulin properly. Diabetes affects people of all ages. Most forms of diabetes are chronic (lifelong), and al forms are manageable with medications and/or lifestyle changes. Diabetes Mellitus is a metabolic disease, involving two categories:– type-1 and type- 2. T1DM is categorised by the destruction of beta cells in the pancreas, typically secondary to an autoimmune process. The result is the absolute destruction of beta cells, and consequently, insulin is absent or extremely low. T2DM involves a more insidious onset where an imbalance between insulin level and insulin sensitivity cause a functional deficit of insulin. Insulin resistance is multifactorial but commonly develops from obesityand aging. Severe hyperglycaemia can lead to various health problems like nerve damage, heart disease and stroke, kidney damage, eye damage, etc. Glucose level monitoring and controlling is thus necessary in patients with diabetes. Traditional method involves needle prick for gaining blood sample followed by checking the respective glucose level. The project is regarding the new method for detection of diabetes mellitus. The new method is without prick and detection of diabetes from just sweat ! As it doesn't involves pricking, patient does not need to bear the pain.

- **INTRODUCTION**

Traditional method involves the detection of DM by using a glucometer, followed by pricking and and blood swab testing within seconds of time.

-Current Methods of monitoring blood glucose level :

1. Invasive method- involves pricking.
2. May cause discomfort and pain.
3. Can cause infection.

-Recent methods of monitoring blood glucose level.

1. Non invasive method
2. involves expensive methods such as –

- Biosensors
- spectrofluorometry

- Cannot be used by Layman
- Complexity of procedure.

- **Principle**

-Glucose is excreted in sweat and correlation exist between blood glucose level and sweat glucose level.

-Glucose oxidase enzyme is glucose specific enzyme and catalyses the oxidation of glucose.

- **Reaction**

Glucose + O2 $\xrightarrow{\text{glucose oxidase}}$ Gluconic acid + H2O2

This enzyme produces H2O2 which is the key reactive component.

- Glucose Oxidase enzyme

Glucose oxidase (GOx), a flavoprotein enzyme, catalyzes the oxidation of β-D-glucose to produce D-glucono-delta-lactone and hydrogen peroxide. It's known for its high specificity for glucose and is used in various industrial and medical applications, including glucose monitoring.

Here's a more detailed breakdown

-Function and Mechanism

Catalysis

GOx catalyzes the oxidation of β-D-glucose to D-glucono-delta-lactone, which then spontaneously hydrolyzes to gluconic acid.

Electron Acceptor

Molecular oxygen acts as the electron acceptor in this reaction, resulting in the simultaneous production of hydrogen peroxide.

FAD Dependence

GOx is a flavoprotein, meaning it contains a flavin adenine dinucleotide (FAD) cofactor, which is essential for its catalytic activity.

Enzyme Class

GOx is an oxidoreductase enzyme.

-Structure

Dimeric Protein

GOx is a dimeric protein, meaning it consists of two identical subunits, each with a molecular weight of around 80 kDa.

Subunit Structure

Each subunit contains a domain that binds to the substrate glucose and another domain that binds to the FAD cofactor.

-Sources

Fungi and Insects: GOx is produced by certain fungi, such as Aspergillus niger and Penicillium notatum, and insects.

Industrial Production: Aspergillus and Penicillium are the main industrial producers of GOx.

-Application

Glucose Monitoring

GOx is used in glucose testing kits and biosensors to detect and measure glucose levels in various samples, including blood and urine.

Food Industry

GOx is used in the food industry to remove glucose from dried eggs and eliminate oxygen from juices and beverages.

Textile Industry

GOx is used in enzymatic pretreatment of textiles. Medical Applications

GOx has antimicrobial effects against various pathogens and is used in medical applications.

Recombinant Production

Recombinant production of GOx is a common approach to produce sufficient amounts of the enzyme for various uses.

-Key Properties

High Specificity: GOx exhibits high specificity for glucose, meaning it primarily catalyzes the oxidation of glucose.

Stability: GOx is known for its high stability.

Fast Mechanism of Action: GOx is known for its fast mechanism of action.

- **Sweat Composition**

Content	Amount
Lactic acid	45-150
Ammonia.	5-10
Urea.	12-60
Phosphorus.	0.2

Sodium chloride. 2000

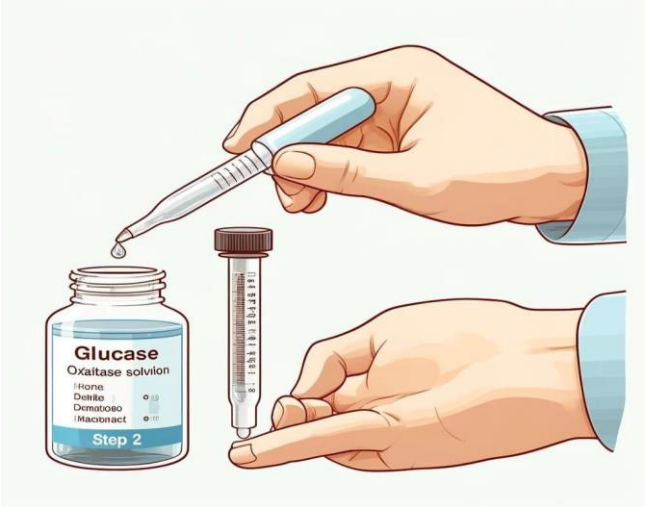
Acetic acid. 12-20

- **Procedure**

- Collect 2 drops of sweat.
- Add 2 drops of glucose oxidase solution
- Add 2 drops of given reagent
- Add 5 drops of starch slurry
- Observe the colour change to bluish brown colour

Step 1: Collection of sweat.

Sweat collection for testing, like in diagnosing cystic fibrosis, typically involves stimulating sweat production with pilocarpine and the collecting the sweat using either the gauze / filter paper method or the capillary tube (Macroduct) method.



Step 2: Preparation of glucose oxidase enzyme.

To prepare a glucose oxidase reagent, dissolve glucose oxidase, perozidase and a chromogen (like odianisidine) in a sodium phosphate buffer, then filter if necessary.

Reagent Stability

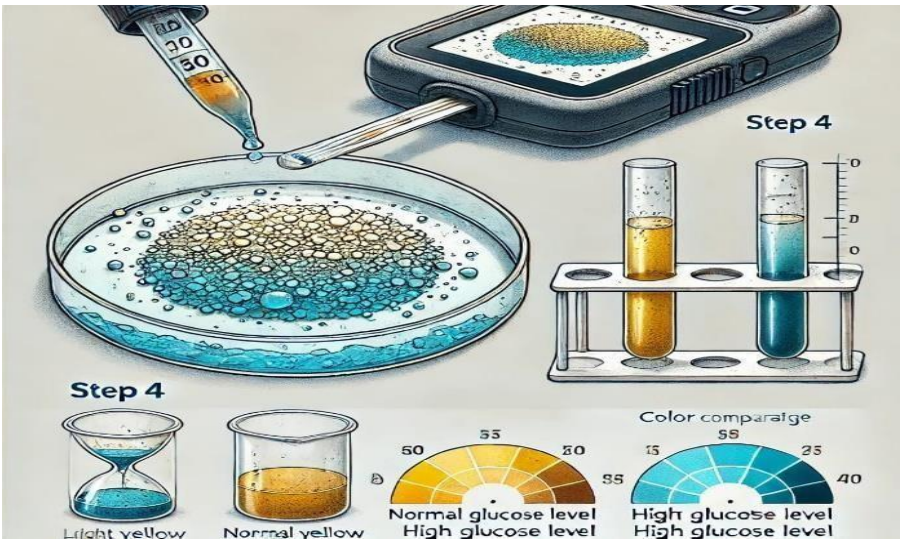
The prepared reagent is active for about 4 weeks if stored in a brown coloured bottle at 4° C.

Step 3

Add 5 drops of starch slurry (To enhance the colour formation).

Here, the starch reagent is acting as a chromogenic reagent and not taking part in reaction. It

just helps to get clear colour change during the process and should not be confused with other reactants and the correlated parts.



Step 4: Observe the colour change to bluish brown, which correlates with glucose level.

- **Correlation Between Blood Glucose Level and Sweat Glucose Level**

There's a strong correlation between sweat glucose and blood glucose level, with sweat glucose being about 1-2 percent of the corresponding blood glucose value, making sweat a potential source for non – invasive glucose monitoring.

Here's a more detailed explanation

Metabolic Relationship

Glucose, a type of sugar, is a primary source of energy for the body, and it's transported through the bloodstream. Sweat glands, which produce sweat, are located throughout the body and are involved in regulating body temperature. Glucose can also be found in sweat, and the levels in sweat are correlated with the levels in the blood.

Sweat Glucose Levels

The concentration of glucose in sweat is significantly lower than in blood, typically around 1-2% of the blood glucose value. Non-Invasive Monitoring:

The correlation between sweat and blood glucose levels has led to research and development of non-invasive glucose monitoring devices that use sweat samples to estimate blood glucose levels.

- **Challenges and Considerations**

Lag Time: There can be a lag time between changes in blood glucose and the corresponding

changes in sweat glucose levels.

Sweat Rate: Sweat rate can affect the concentration of glucose in sweat, potentially influencing the accuracy of glucose monitoring.

Skin Surface Glucose: Glucose on the skin's surface can contaminate sweat samples, leading to inaccurate readings.

- **Advantages**

-The method is non invasive .As the diabetes patients are very sensitive to infection and healing in diabetics is slow process, the method is beneficial for them.

-It is painless.

- The method gives rapid result. Hence not lag as compared to glucometer in relative of tim
- The procedure is simple and thus, does not require special technicians and can carry out at household as well.
- The process is economical hence affordable to financially instable patients also.

- **Precautions**

Though, the procedure is simple and can carry out at household, it is crucial to take some precautions during the procedure. Those are a as follows :

- Keep the solutions in a cool, dry place and away from sunlight.
- Do not add excess of solutions to the sweat.
- Keep an interval of 5 sec after the addition of each solution.
- Maintain the hygiene by washing hands or using gloves during and after collection of sweat.

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