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Development of Potentiometric Urea Biosensor For Clinical Purposes

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ABSTRACT: This review summarizes the studies carried on the development of potentiometric urea biosensor in laboratory using urease from *Cajanus cajan* (local Arhar). The enzyme used was free without any immobilisation over matrix. The electrode used egg membrane or cellophane paper making ionic diffusion possible across the two solutions. Underlying the importance of this study is the fact that urea is toxic above certain concentrations in different biological samples and its continuous real time monitoring in clinical environments is of utmost importance. The conventional analytical techniques used, although precise is time- consuming and laboratory bound whereas biosensors have the advantages of ease of use, portability and the ability to furnish real time signals. © 2011 IGJPS. All rights reserved.

KEYWORDS: Potentimetric Urea Biosensor; Clinical Utility; *Cajanus cajan*; Arhar.

INTRODUCTION

Urea is an important biomolecule to humans. It is formed as a result of urea cycle in body from ammonia or by oxidation of amino acids. It is excreted in urine or in sweat by humans. Our body fluids contain a fixed amount of urea. Any discrepancies in its normal level may be toxic to us. Hence, its constant real time monitoring should be in use for clinical purposes.

PRINCIPLE:

The urease was extracted from *Cajanus cajan* according to the method described by Kayastha and Das in 1999. After extraction of urease, a potentiometric electrode containing 0.5 M Copper (II) sulphate was placed in the sample containing urease. By the action of the enzyme, the urea in the sample breaks into ammonium ions and carbonate ions. Ammonium ions being highly soluble in aqueous medium render it alkaline. This creates an electrochemical gradient due to which ionic diffusion is possible across the semi permeable membrane. This potential difference is measured using a voltmeter.

MATERIALS & METHODS

MATERIALS:

50 gms of pigeonpea seeds, 100 ml of 0.025M and 0.05M Tris-acetate buffer (pH 6.5), few drops of leupeptin, 32% acetone, centrifuge tubes, centrifuge, distilled water, test tubes, coffee grinder, mortar and pestle, 10 ml 0.5 M Copper (II) sulphate solution,

pipette, egg membrane, cellophane paper, digital voltmeter, rubber band, pair of insulated copper wires, blade, broken test tube, buffer with pH 4, Biuret reagent.

METHODS:

(i) EXTRACTION OF UREASE FROM *CAJANUS CAJAN*-

Pigeonpea seeds were bought from a local vendor. 50 gms of seeds were soaked overnight at $4^{o}C$ in Tris-acetate buffer because of maximum extractable capacity and restoration of maximum enzymatic activity. Seeds were ground in the extraction buffer with few drops of leupeptin in a coffee grinder the next morning and the temperature was restored to $4^{o}C$. Leupeptin is added to denature any disulphide containing peptidase that might be present so as to harm the required enzyme. Then the paste was filtered twice using muslin cloth. The filtrate was centrifuged at 5000 rpm for 15 minutes. The temperature was immediately maintained at $4^{o}C$. Pre-chilled acetone was added to the clear supernatant to one-third of the volume of extract with constant whirling movements. After 15 minutes of interval, the suspension was centrifuged at 5000 rpm again for 2 minutes. Washing with acetone was again repeated once in the same way as before. The precipitate was then collected and dissolved in 0.05 M Tris- acetate buffer (pH 6.5) and stored at $<2^{o}C$ in freezer which retains its activity for about 3 months. Biuret test is then performed on the precipitate to test the presence of enzyme. Later, change in pH was determined to check the biological activity of enzyme.

(II) ASSEMBLING THE POTENTIOMETRIC UREA BIOSENSOR-

A broken test tube was sealed at one end by a cellophane paper or an egg membrane using rubber bands. 10 ml of 0.05 M Copper (II) sulphate solution was poured into it using a pipette. A wire with one end exposed went into the test tube and its other end went into the negative terminal of the voltmeter. Similarly, another wire with one of its exposed ends was immersed in the biological sample and the other end was connected to the positive terminal of the voltmeter. When not in use, the electrode was immersed in the beaker containing buffer of pH 4. The electrode was wiped with a dry tissue paper before use.

Then, 2 ml of urease was poured in the 10 ml of sample and after 2 minutes the electrode was placed in the test solution to measure voltage.

RESULTS & DISCUSSION

The sensitivity of the urea biosensor was detected to be 25×10^{-5} M. The potential difference readings in saliva sample were found to be erratic due to unknown reasons. Urine and blood were taken both from males and females(Table 1 & Table 2). So, the normal range of potential difference for urine came out to be 0.194V to 0.244V whereas for blood it was around 0.150V to 0.172V. The readings taken using egg membrane and cellophane paper lay in a similar range but cellophane paper was preferred due to increased durability.

S. No.	Response time (in minutes)	Potential Difference (in volts)
1.	2	0.235
2.	2	0.197
3.	2	0.220
4.	2	0.240
5.	2	0.237

Table 1 Potential difference readings in urine sample

S. No.	Response time (in minutes)	Potential Difference (in volts)
1.	2	0.168
2.	2	0.153
3.	2	0.171
4.	2	0.165
5.	2	0.159

Table 2 Potential difference readings in blood sample

CONCLUSION

Urease was extracted from *Cajanus cajan* (pigeonpea). There was considerable change in pH of the biological sample indicating the potency of the enzyme. The pH biosensor was designed for quantitative estimation of the presence of urea in blood and urine sample.

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