Research Article

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POTENTIOMETRIC BIOSENSOR FOR ASPARAGINE DETECTION

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ABSTRACT

Catharanthus roseus is a plant of medical value and has been used since many years. It gives a number of alkaloids which imparts the medicinal importance from this plant. L-asparaginase enzyme is produced by bacteria, fungi, yeast, actinomycetes and plants. L-asparaginase is known to treat cancer by breaking down asparagine to aspartic acid and ammonia. Using L-asparaginase extracted from fresh leaves of *Catharanthus roseus* a potentiometric biosensor was developed. The crude enzyme was immobilized in different matrixes such as calcium alginate beads, agar and paraffin wax. The immobilized enzyme was conjugated with Ion sensing electrode (ISE) and its mV readings were noted with different asparagine concentrations ranging from 10^{-9} to 10^{-1} M. Calcium alginate beads gave the most reliable response so they were used for the detection of asparagine levels in leukemic serum samples. The asparagine concentration in leukemic serum samples was 10^{-2} to 10^{-3} M. The developed biosensor was reliable, novel, cost effective and easy to use.

Keywords: *Catharanthus roseus*, L-asparaginase, calcium alginate beads, agar, paraffin wax, leukemic serum samples, asparagine.

INTRODUCTION

L-asparaginase is an enzyme used for the treatment of different types of leukemia such as Acute Lymphoblastic Leukemia, lymphosarcoma, mylenosarcoma, etc. It is a tetrameric protein and is produced by a number of sources such as micro-organisms and plants. It catalyzes the hydrolysis of asparagine to aspartic acid and ammonia[1]. Asparagine is non-essential amino acid which is synthesized by the cells on their own by enzyme asparagine synthetase[2]. But the cancer cells lack this ability of synthesizing asparagine and hence depend on the extracellular supply. If L-asparaginase is injected into the blood it breakdown asparagine to aspartic acid and ammonia due to which the cancer cells die. Lang[3] was the first person who found its activity in beef tissues which was then confirmed by Furth and Friedmann[4]. Some of the microbial sources are *Escherchia coli*, *Erwinia carotovora*[5], *Proteus pseudomonas*, *Aerobacter*, *Vibrio serratia*, *Aspergillus*, *Xanthomonas* and *Bacillus*[6-8], whereas the plant sources include *Lupinus luteus*, *Dolichos lab lab*[9], *Pisum sativum*[10] and *Tamarindus indica*[11]. In case of plants L-asparaginase is accumulated under the stress conditions and used as nitrogen storage as well as transporting compound[12]. For the detection of asparagine the use of biosensors is an efficient alternative. Among the various types of biosensors plant biosensors are chosen because they give fast, quick and efficient response. For monitoring the levels of hydrogen peroxidase a biosensor was developed using the enzyme extracted from *Allium sativum* and immobilizing it on chitosan matrix[13]. Acetaminophen was detected using the poly phenol oxidase isolated from banana tissues and immobilized on poly pyrrole matrix[14]. Oxalate oxidase extracted from barley root was immobilized on hybrid of gold nanoparticles and porous $CaCO_3$ for the development of amperometric oxalate biosensor[15]. Biogenic amine was detected in beer and wine samples by using an electrochemical biosensor made by immobilizing diamine oxidase extracted from *Lathyrus sativus*[16]. *Catharanthus roseus* (Voucher number PTBG0000045052) is a medically important plant used as anti-diabetic, anti-cancer, anti-mutagenic and anti-microbial agent. In the present study a potentiometric biosensor was constructed by immobilizing enzyme extracted from *Catharanthus roseus* on different matrixes. ISE (Ion Sensing Electrode) was used for the detection of asparagine levels in leukemic serum samples.

MATERIALS AND METHODS

Extraction of Crude Enzyme

All the chemicals and reagents used in this study were of analytical grade (Hi-Media Laboratories Pvt. Ltd., India.). The plant, *Catharanthus roseus* was collected from different regions of Punjab, India. Fresh leaves were washed with distilled water and homogenized with 3 volumes of 0.15M KCl and centrifuged at 8000 rpm for 20 minutes at 4˚C. The supernatant was separated out; this was designated as crude extract[11] and further used for the development of biosensor. In biosensor construction strategies, L- asparaginase was co-immobilized with calcium alginate, agar as described by Kumar *et al*., 2012[17] and paraffin wax.

Immobilization Techniques
Calcium alginate beads

Slurry of 3 % sodium alginate with 20 µl of the enzyme solution (0.5 U) was formed. This solution was then poured drop wise through a glass syringe into a beaker containing 0.075 M chilled $CaCl_2$ with gentle stirring on a magnetic stirrer. Pale color beads were made with the help of 2.5 ml syringe without needle following the method of Johnsen and Flink[18]. Harden the beads by placing it for half an hour at room temperature. Detection limit of L-asparagine achieved was 10^{-9} – 10^{-1} M. NH_4^+ Ion Sensing Electrode (ISE) is used for quantitative analysis.

Agar method

A solution of 4 % agar was prepared, boiled it and allowed to cool at 40-45˚C. 20 µl enzyme (0.5 U) was added to the solution. Mixed it thoroughly and poured it into 90 mm petriplate and allowed to solidify. The gel was then cut into square cakes of 1.0 x 1.0 cm with the help of knife as described by Mahajan *et al*., 2010[19]. Detection limit of L-asparagine achieved was 10^{-9}-10^{-1} M. NH_4^+ Ion Sensing Electrode (ISE) is used for quantitative analysis.

Paraffin Wax

4 g of paraffin wax was melted in a thermostated water bath and 20 µl enzyme (0.5 U), sundried and then cut into small pieces of 1 cm^2. After 2-3 sec the pieces were removed with the help of forceps and dried at room temperature[20]. Detection limit was observed using NH_4^+ ISE.

Monitoring of Asparagine Levels in Normal and Leukemia Blood Serum Samples

The beads of calcium alginate, agar cakes and paraffin wax blocks were put into normal and leukemia blood samples. The asparagine levels in both the samples were monitored.

Check the Reliability of the NH_4^+ISE

To check the reliability of the ISE, calculation of Δ mV was studied by formula:

$$1/2x + 1/2y = X$$

Where x = Serum sample and y = Synthetic sample of L-asparagine

Storage Stability

To know the storage stability of biocomponent i.e. agar cakes, paraffin wax blocks and calcium alginate beads were wrapped in a Whatman filter paper soaked in $CaCl_2$ and were kept in refrigerator. The activities of immobilized bio components were checked.

RESULTS AND DISSCUSION
Calcium Alginate Beads

Detection limit of L-asparagine achieved was 10^{-9}-10^{-1} M. For concentration level of 10^{-1} M L-asparagine, the mV reading detected was -196.5. The range of mV reading falls between -236.1 to -196.5 (Figure 1). With decrease in the asparagine concentration the reading also decreases. Calcium alginate beads method gave the fastest and most stable response.

Agar Method

With 10^{-1} M concentration of asparagine the mV reading was -50.0 and with 10^{-9} M it was -84.2 (Figure 1). Detection limit of L-asparagine achieved was 10^{-9}-10^{-1} M.

Paraffin Wax

The mV readings fall in the range of -53.0 to -35.5 as shown in Figure 1 and the detection range of asparagine was 10^{-9} to 10^{-1} M. The readings decrease with the decrease in asparagine concentration.

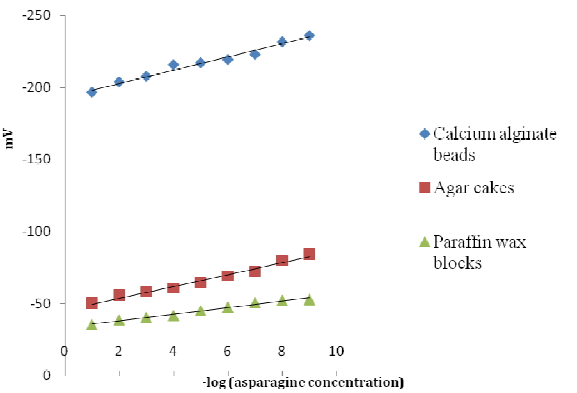

Figure 1: Standard curve of asparagine using different immobilization techniques

Testing Asparagine Levels in Normal and Leukemia Blood Serum Samples

Out of the various immobilization techniques applied calcium alginate beads gave the most reliable response so it was further used for the detection of asparagine levels in leukemic serum samples. For the leukemic serum samples the asparagine concentration was 10^{-2} to 10^{-3} M and for the normal cells it was 10^{-5} to 10^{-6} M.

Reliability Check for the Constructed Biosensor

The developed biosensor was found to be comparable and reliable.

Storage Stability of the Bio component

The biocomponent was found to be active. Paraffin wax blocks, agar cakes and calcium alginate beads were found to be stable for a long time i.e. more than fifteen days, fifteen days and four months respectively.

Catharanthus roseus is therapeutically important plant known for its anti-tumor, anti-mutagenic, anti-diabetic, anti-oxidant and anti-microbial activity[21]. The detection range of the developed biosensor was 10^{-9}-10^{-1} M and it was further used for the detection of asparagine levels in leukemic serum samples. In earlier work carried out in our laboratory different biosensors were developed using extracts of *Capsicum annum*[22], *Withania somnifera*[17], *Citrus lemon*[23] and *Cannabis sativa*[24]. In these biosensors visual approach was used to measure the response time as well as the levels of asparagine in cancer samples. The enzyme used was 0.5 U which makes the biosensor cost effective, rapid, easy to use and reliable.

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