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Digital Sensors and Sensor Systems: Practical Design

Sergey Y. Yurish



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Topic E2: Transportation & Mobility

The Euromat conference series, organised by the Federation of European Materials Societies (FEMS), is one of the largest events of its kind in Europe, covering the full width of materials science and technology. We would like to direct your attention to the following Symposia which are focussing specifically on transport applications:

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Prof. Kambiz Kayvantash, Société CADLM, Massy (F)

E2.II: **Intelligent and adaptive materials and structures**
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L-Asparaginase Extracted From *Capsicum annum L* and Development of Asparagine Biosensor for Leukemia

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Abstract: Green chillies (*Capsicum annum* L.) contain appreciable amount of L-asparaginase enzyme and the present work aims at the development of asparagine biosensor for leukemia. It is a novel and diagnostic plant based biosensor for monitoring asparagine levels acute lymphoblastic leukemia (ALL) samples. Different immobilization techniques and response time studies have been carried out to improve the stability of enzyme by these methods. Phenol Red indicator has been coimmobilized with plant asparaginase and color visualization approach has been optimized for various asparagine ranges. For quantitative analysis, immobilized biocomponent is coupled to Ion Sensing Electrode (ISE) of a potentiometeric transducer. The detection limit of asparagine achieved with immobilization techniques such as gelatin, polyacrylamide gel, agar and calcium alginate beads method is 10^{-1} –10^{-9}M. Also, these techniques have been applied for the detection of asparagine in normal (10^{-4}M) and leukemia blood serum samples (10^{-2}M). *Copyright © 2012 IFSA.*

Keywords: *Capsicum annum,* L-asparaginase, Leukemia, Biosensor, Immobilized.

1. Introduction

The enzyme L-asparaginase is widely used as antitumoral agent for the treatment of acute lymphoblastic leukemia (ALL) [1]. L-asparaginase is the first enzyme which is to be studied in human beings because of its antitumour activity [2]. Tumor cells need L-asparagine for their growth because they lack the enzyme, asparagine synthetase that synthesizes this amino acid [3] and in the presence of L-asparaginase, tumor cells cannot survive due to unavailability of important growth factor [4]. Lang was the first person to detect asparaginase activity in beef tissues [5]. Kidd reported antitumour properties of Guinea pig serum, which was later attributed to asparaginase activity [6-7]. Thus ALL

and lymphosarcoma are effectively treated by L- asparaginase and has been clinically accepted as antitumour agent [8].

The enzyme L-asparaginase is produced by number of microorganisms such as *Erwinia cartovora* [9], *E. coli* [10], *Candida utilis* [11], and *Thermus thermophilus* [12]. Among various plants species, production of L asparaginase has been reported. Green chillies *(Capsicum annum L.)* contained L- asparaginase enzyme, its extraction partial purification and properties of L- asparaginase from green chillies has been reported [13]. *Withania somnifera* was identified as potential source of L-asparaginase due to high specific activity [14]. The presence of an amidase in barley roots capable of hydrolyzing L-asparagine [15]. L-asparaginase was also produced in large amount from root nodules of soyabean [16]. *Pisum sativum* contain appreciable amount of L-asparaginase and was also detected in young leaves [17]. L-aparaginase activity was also detected from two more plants *Lupin arboreus* and *L.angustiplius* [18].

For the fabrication of biosensor, an online gas analyzer for automated enzymatic analysis with potentiometric ammonia detection has been developed [19]. *E. coli* K-12 asparaginase based L- asparagine biosensor has been developed to detect asparagine levels in normal and leukemia blood samples [20]. An amperometric biosensor based on spinach (*Spinacia aleracea*) tissue homogenate was developed for determination of urinary oxalate [21]. L- asparaginase was immobilized and used with an ammonium selective electrode (ISE) to develop enzymatic biosensor. Garlic tissue electrode is used to determine L-asparagine where garlic tissue cells were employed for conversion of L-asparagine into ammonia and ammonium gas electrode was used as detector [22]. A thermostable recombinant asparaginase from *Archaeoglobus fulgidus* was cloned and expressed in *E.coli* as a fusion protein and the immobilized enzyme was used with an ammonium selective electrode to develop a biosensor for L-asparaginase [23]. A garlic (*allium sativum L.)* peroxidase biosensor for hydrogen peroxidase monitoring, which was immobilized on chitosan matrix was formed [24]. P*etunia punctata, Alternanthera sessilis & Amoora chittagonga* extracts showed cytotoxicity screening against three pancreatic cancer lines-adenocarcinoma cell line Panc-1, Mia-Pacca-2, capan-1 using label free biosensor assay [25]. Amperometric oxalate biosensor based on sorghum leaf oxalate oxidase, immobilized on carboxylated multi-walled carbon nano tubes and conducting polymers, polyaniline composite film was constructed [26]. The current study presents the development of a novel, diagnostic and cost-effective plant L-asparaginase based biosensor for leukemia blood samples.

2. Experimental

2.1. Biological Materials and Reagents

All the chemicals and reagent used in the study were of analytical grade. The transducer is a benchtop potentiometer (Cyberscan 2500) in conjunction with an NH_4^+ ion selective electrode (ISE Code No. EC-NH4-03) that detects the electrode potential developed across the membrane of the electrode when it comes in contact with NH_4^+ ions.

2.2. Crude Extract Preparation

The enzyme L-asparaginase was extracted from green chillies (*Capsicum annum* L.). Fresh green chillies (about 250 g) were homogenized with three volumes of 0.15 M KCl buffer solution and centrifuged at 4 ^{0}C. The supernatant was separated out and the pellet formed was dissolved and re-extracted with 0.15 M KCl buffer solution [13]. The obtained supernatant was designated as crude extract (L-asparaginase) and further for biosensor applications, it was coimmobilized with phenol red

indicator (HiMedia Laboratories Pvt. Ltd., India.) that changes color from red to violet (red color was due to phenol red indicator). When the enzyme L-asparaginase come in contact with substrate (asparagine), deamination reaction occur in which asparagine break down into aspartic acid and NH_4^+ ions and color change response was observed. Asparagine concentrations ranging from 10^{-9}-10^{-1} M were studied by color visualization approach and with potentiometeric transducer. Immobilization techniques for the biosensor fabrication are as follow.

2.3. Gelatin Method

2 gm of gelatin was dissolved in water by heating it properly. After cooling to 35-40 ^{0}C, 20 µl enzymes (0.5U) were co-immobilized with 10 µl phenol red indicator. 2 ml of hardening solution was added comprising of 4ml formaldehyde, 6 ml water and 10 ml ethanol. It was allowed to freeze at -20 ^{0}C for 4 hour to facilitate the gel formation. Then gel was warmed to room temperature and cut into square blocks of 1.0 × 1.0 cm [27]. Put these blocks into varying concentration of L-asparagine and the response time was noted for change in color of blocks from partly orange to dark purple. For quantitative analysis, NH_4^+ Ion Sensing Electrode (ISE) is used.

2.4. Polyacrylamide Method

A 10 % acrylamide and bis-acrylamide solution (9 % acrylamide and 1% bis- acrylamide) was prepared in 0.1 M phosphate buffer (pH 7.0). 20 µl of enzyme (0.5U) was coimmobilized with 10 µl phenol red indicator in acrylamide solution. To the above solution 0.5 gm of ammonium per sulphate was added. 50 ml TEMED was added and contents were stirred gently and the solution was poured into petriplate. After it was solidified the gel was cut into square blocks of 1.0 × 1.0 cm [28]. The pieces of gel were taken in different concentration of L-asparagine and the color change was noted down. Then the pieces of gel were then put into varying concentrations of L-asparagine and note the reading with NH_4^+ ISE.

2.5. Agar Method

The agar solution of 4 % concentration was heated to liquefy the agar and allowed to cool at 45-50 ^{0}C. 20 µl enzyme (0.5U) and 10 µl phenol red indicator was added to the solution. The contents were stirred gently, poured it into petriplate and allowed it to solidify. The gel was then cut into square cakes of 1.0 × 1.0 cm with the help of knife or spatula [29]. Then cakes were put into varying concentration of L-asparagine and the response time was noted for change in color of cakes from partly orange to dark purple. Detection limit of L-asparagine achieved was 10^{-9} – 10^{-1} M. NH_4^+ Ion Sensing Electrode (ISE) is used for quantitative analysis.

2.6. Calcium Alginate Beads

It was carried out by Sodium alginate $CaCl_2$ technique. Slurry of 3 % sodium alginate with 20 µl of the enzyme solution (0.5U) was formed and 10 µl of phenol red indicator were added to this slurry. 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. Orange color beads (partly orange color of the beads was due to phenol red indicator) were made with the help of 2.5 ml syringe without needle [30]. The beaker was kept for half an hour for hardening of the beads. Beads were then washed with distilled water for further use. The beads were put into varying concentrations of L-asparagine (10^{-9}-10^{-1} M) solutions. The response time for change in color of beads from partly orange to bright purple was

noted. For quantitative analysis, the beads were then put into varying concentrations of L-asparagine was noted with NH_4^+ Ion Sensing Electrode (ISE) of a potentiometeric transducer.

2.7. Monitoring of Asparagine Levels in normal and Leukemia Blood Serum Samples

The beads of calcium alginate were put into normal and leukemia blood samples. Response time for color change of beads till purple color appears was noted. The asparagine levels in both the samples was monitored by relating the response time for change in color of beads of both the samples with the response time for change in color of beads with concentration levels from 10^{-9} – 10^{-1} M of asparagine.

2.8. Check the Reliability of the NH_4^+ISE

To check the reliability of the ISE, calculation of Δ mV and response times for change in color was studied by formula:

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

where x = Serum sample and y = Synthetic sample of L-asparagine.

2.9. Storage Stability

To know the storage stability of biocomponent i.e. gelatin gel blocks, polyacrylamide gel, agar cakes and calcium alginate beads were wrapped in a Whattmann filter paper soaked in $CaCl_2$ and were kept in refrigerator. The activities of immobilized biocomponents were checked.

4. Results and Discussion

4.1. Gelatin Method

For gelatin method, visual color change was observed. The comparison of color of gel blocks before and after the reaction (see Fig. 1). Detection limit of asparagine achieved was 10^{-9} – 10^{-1} M. For concentration level of 10^{-1} M L-asparagine, response time and mV reading detected was 22 seconds, -135.2 and for the concentration level of 10^{-9}-10^{-2} M L-asparagine, response time detected was in the range of 10-20 seconds (Table 1). Response time decreased with decreased in concentration of asparagine indicating more of NH_4^+ ion produced after hydrolysis**.**

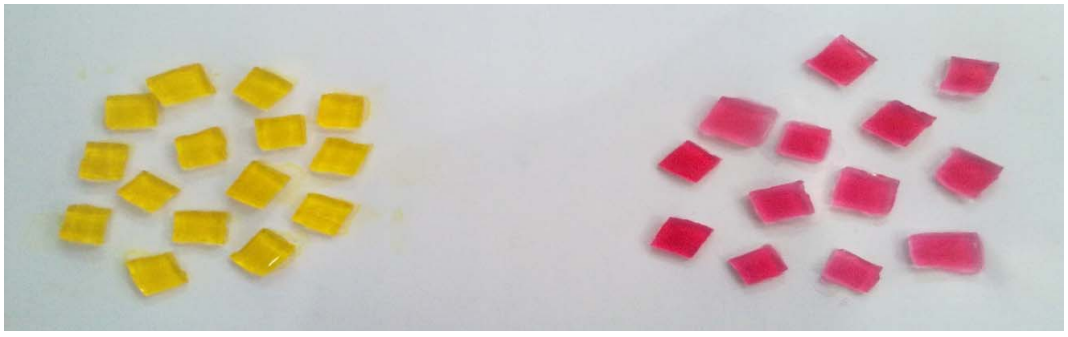


Fig. 1. Comparison of Color of gel blocks (Before and after the reaction).

Table 1. The mV readings with NH_4^+ ISE and response time.

Concentration of L-asparagine, (Molar)	Potential Value, (mV)	Response Time, (s)
10^{-1}	-135.2	21.6
10^{-2}	-147.7	20.0
10^{-3}	-159.9	17.9
10^{-4}	-170.2	16.5
10^{-5}	-179.1	15.6
10^{-6}	-183.7	14.0
10^{-7}	-195.6	12.3
10^{-8}	-204.2	11.3
10^{-9}	-210.8	10.0

4.2. Polyacrylamide Method

Detection limit of L-asparagine achieved was 10^{-9} – 10^{-1} M. For concentration level of 10^{-1} M L-asparagine, response time and mV reading detected was 20 seconds, -147.7 and for the concentration level of 10^{-9}-10^{-2} M L-asparagine, response time detected was in the range of 10-19 seconds (Table 2). Response time decreased with decreased in concentration of L-asparagine indicating more of NH_4^+ ion produced after hydrolysis (see Fig. 2).

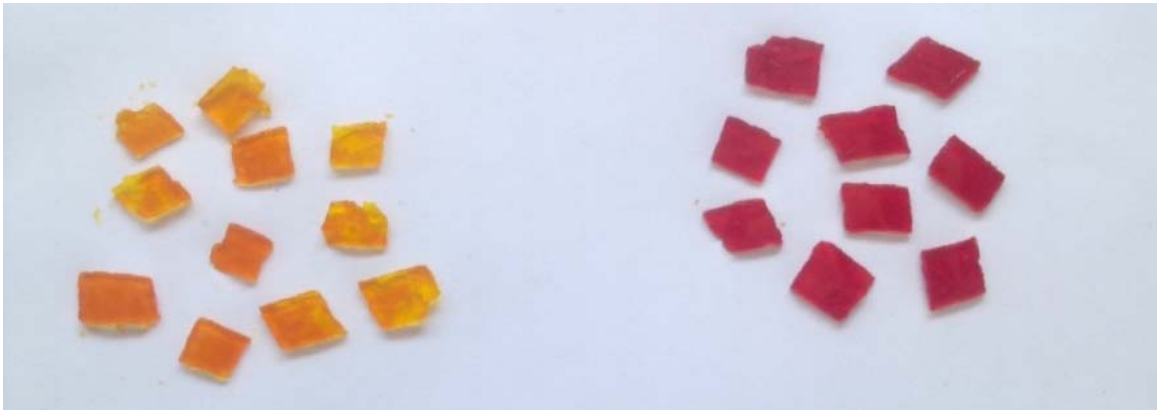


Fig. 2. Comparison of Color of gel pieces (Before and after the reaction).

Table 2. The mV readings with NH_4^+ ISE and response time.

Concentration of L-asparagine, (Molar)	Potential Value, (mV)	Response Time, (s)
10^{-1}	-147.7	20.0
10^{-2}	-156.6	18.7
10^{-3}	-170.5	16.6
10^{-4}	-178.0	15.3
10^{-5}	-183.9	13.9
10^{-6}	-188.2	13.1
10^{-7}	-204.2	11.3
10^{-8}	-207.5	10.5
10^{-9}	-210.8	10.0

4.3. Agar Method

Detection limit of L-asparagine achieved was 10^{-9} – 10^{-1} M. For concentration level of 10^{-1} M asparagine, response time and mV reading detected 14 seconds, -180 and for the concentration level of 10^{-9}-10^{-2} M, L-asparagine response time detected was in the range of 7-13 seconds (Table 3). With decrease in concentration of asparagine, the response time for color change increases (see Fig. 3).



Fig. 3. Comparison of Color of gel blocks.

Table 3. The mV readings with NH_4^+ ISE and response time.

Concentration of L-asparagine, (Molar)	Potential Value, (mV)	Response Time, (s)
10^{-1}	-180.0	14.2
10^{-2}	-196.5	12.5
10^{-3}	-204.0	11.2
10^{-4}	-208.7	11.0
10^{-5}	-212.4	9.8
10^{-6}	-217.1	8.8
10^{-7}	-220.3	8.5
10^{-8}	-226.7	8.0
10^{-9}	-230.2	7.5

4.4. Calcium Alginate Beads

For calcium alginate beads, visual color change was observed. The comparison of color of beads before and after the reaction (see Fig. 4). Detection limit of L-asparagine achieved was 10^{-9} – 10^{-1} M. For concentration level of 10^{-1} M L-asparagine, response time and mV reading detected was 12 seconds, -195.5 and for the concentration level of 10^{-9}-10^{-2} M L-asparagine, response time detected was in the range of 7-11 seconds (Table 4). This is directly related to decrease in NH_4^+ produced after the reaction due to increasingly lesser concentration of the reactant. In the comparison of all immobilized techniques, calcium alginate beads method is fast time response and more stable.



Fig. 4. Color of beads before and after the reaction.

Table 4. The mV readings with NH_4^+ ISE and response time.

Concentration of L-asparagine, (Molar)	Potential Value, (mV)	Response Time, (s)
10^{-1}	-195.5	12.3
10^{-2}	-204.6	11.2
10^{-3}	-208.0	10.5
10^{-4}	-213.7	9.4
10^{-5}	-216.1	9.0
10^{-6}	-218.0	8.5
10^{-7}	-223.5	7.8
10^{-8}	-230.9	7.5
10^{-9}	-235.0	7.1

4.5. Testing Asparagine Levels in Normal and Leukemia Blood Serum Sample by Calcium Alginate Beads

Response time for change in color of the beads was 9.4 seconds for the normal blood serum sample and the asparagine concentration level was in the range of 10^{-4}M. Response time for change in color of the beads was 11.2 seconds for the leukemia blood serum sample and the asparagine concentration level was 10^{-2} M. Thus, asparagine levels were found to be high in leukemia blood than normal blood.

4.6. Reliability Check for the Constructed Biosensor

The response times for color change 10^{-2} M and 10^{-4} M was done to check reliability of the developed biosensor. Hence, the developed biosensor is quite reliable and comparable. Hence, visualization approach and ISE transducer coupling can be opted for monitoring L-asparagine concentration in blood samples of leukemia and normal samples.

4.7. Storage Stability of the Biocomponent

The biocomponent was found to be active. Gelatin gel blocks, Polyacrylamide gel pieces, agar cakes and calcium alginate beads were found to be stable for a long time i.e. more than fifteen days, one month, fifteen days and four months respectively.

5. Conclusion

The developed biosensor using various immobilization techniques was able to detect asparagine levels from 10^{-9}-10^{-1} M and further it was used for detection of asparagine levels in normal and leukemia blood serum samples. In comparison with the asparagine biosensors developed by Fraticelli and Meyerhoff and Wang, asparagine range of 10^{-9} M could be detected while the earlier efforts could detect levels up to only 10^{-5} M [19, 23]. Moreover, the unit of enzyme used for the detection of asparagine levels is about 0.5 U. Thus, rapid detection of asparagine concentrations by minute quantities of enzyme (20 µl) is possible that makes the biosensor extremely cost-effective. Thus, the developed biosensor is novel, diagnostic, very rapid, easy to use, inexpensive, portable and capable of nanolevel asparagine detection.

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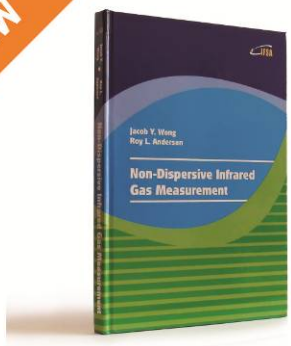
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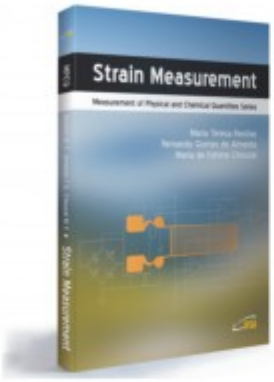
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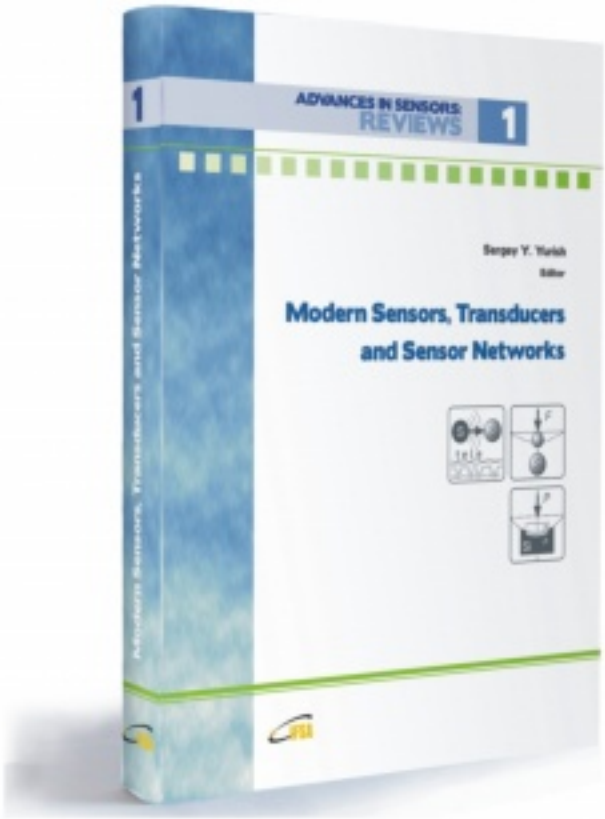
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