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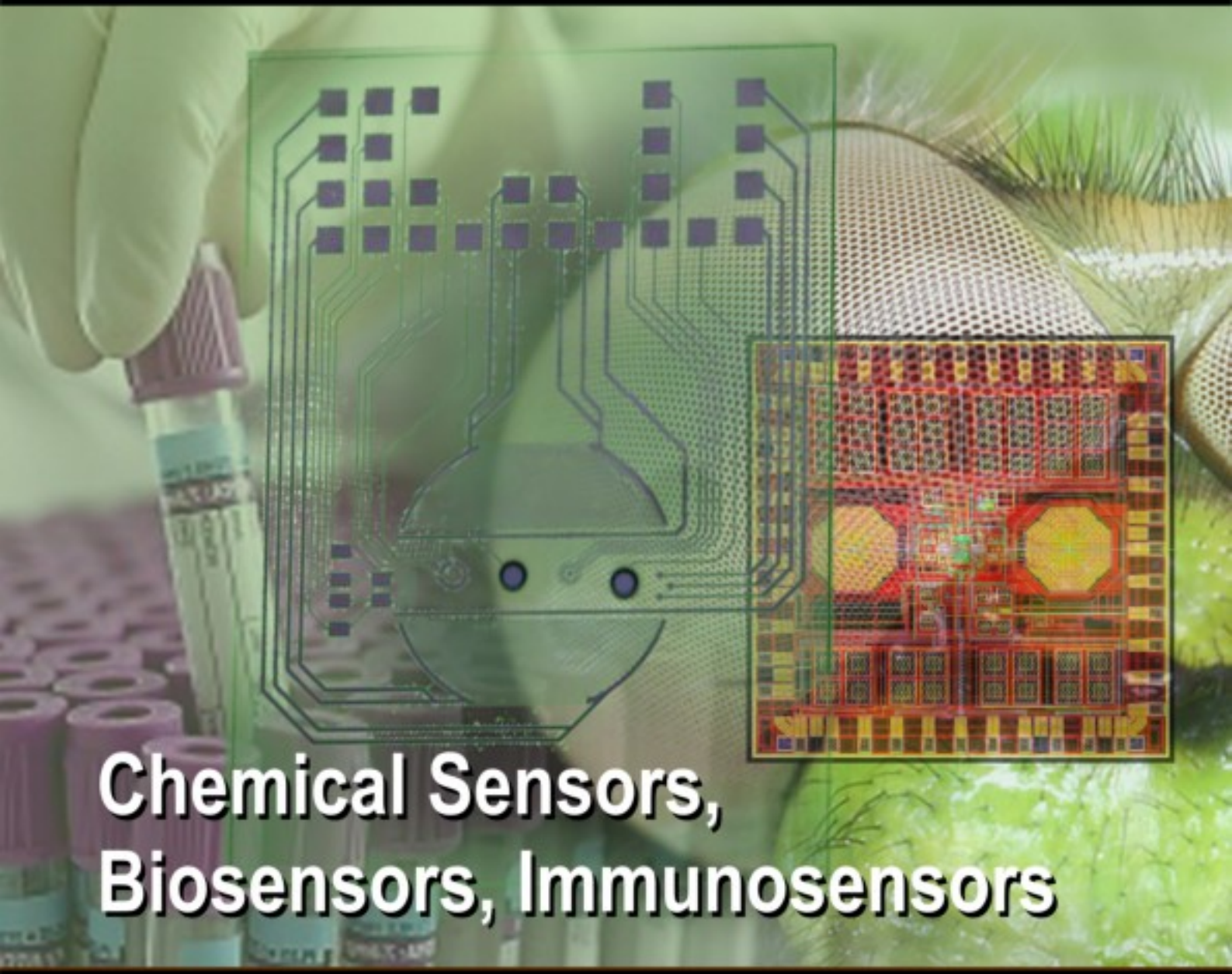

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An Electrochemical Oxalate Biosensor Based on CA Membrane Bound Sorghum Oxalate Oxidase

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Abstract: A method is described for construction of an electrochemical oxalate biosensor using CA membrane bound sorghum leaf oxalate oxidase The biosensor showed optimum response within 2 min at 0.65V, pH 5.5 and 35 °C. The biosensor was employed for amperometric determination of oxalate in urine was. The minimum detection limit of the method was 0.02mmoles/L of urine. Analytical recovery of added oxalate was 95.9 %. Within and between assay coefficients of variation were <3.1% and <6.5 % respectively. A good correlation (r=0.982) was found between oxalate values obtained by a modified Sigma kit method and the present biosensor. The enzyme electrode lost 50 % of its initial activity after its 100 uses over a period of 30 days, when stored at 4 °C. The electrode has the advantage over barley enzyme electrode, as it is free from interference by Cl^- and NO_3^- normally found in biological fluids. *Copyright © 2010 IFSA.*

Keywords: Oxalate, Oxalate oxidase, Oxalate biosensor, Urine, Sorghum leaf, CA membrane,

1. Introduction

The determination of oxalate in urine and plasma is very important in the diagnosis and medical management of calcium oxalate urolithiasis, primary and secondary hyperoxaluria, ethylene glycol poisoning, steatorrhoea and malabsorption [1]. A number of non-enzymic methods such as titrimetric, colorimetric, GLC isotachophoresis, isotope dilution and mass spectrometry, ion chromatography, HPLC and enzymic methods such as enzymic spectrophotometric and enzymic colourimetric [1-2] and enzyme electrodes [3-15]are available for oxalate determination. Enzyme electrodes are comparatively

simpler, sensitive, specific, and reads the sample instaneously. Electrochemical enzyme electrodes based upon generation of e^- from H_2O_2 represent the most successful type of biosensing. [7-15]. The cellulose acetate (CA) permselective barrier confers the required selectivity for H_2O_2 over the interferent constituents of urine (e.g. ascorbate, urate and glutathione) [8].However only barley oxalate oxidase immobilized onto different supports has been employed in such electrodes, which is sensitive to Cl^- and NO_3^- normally found in biological fluids. [16-18]. Thus the enzyme electrode based on barley enzyme requires pretreatment of urine sample for removal of these interferering ions, before analysis which complicate the procedure. This problem was overcome in the present electrode system employing a CA membrane bound Cl^- and NO_3^- insensitive oxalate oxidase purified from grain sorghum leaves.

2. Experimental Section

2.1. Chemicals and Biological Material

Sephadex G-200, DEAE-Sephacel, oxalic acid, methyl viologen 4-aminophenazone, and polyvinyl polypyrrolidone, cellulose acetate (CA) and bovine serum albumin (BSA) (fraction IV) were from Sigma Chemical Co., USA .Acrylamide, bisacrylamide, silver nitrate, TEMED, tris, glycine, EDTA, FC reagent.Phenol, $(NH_4)_2SO_4$ (enzyme grade) from SISCO Research Lab Pvt. Ltd, Mumbai. Pt and Ag/AgCl electrodes from Toshniwal Process Instruments. Pvt. Ltd, Ajmer, were used. Seeds of grain Sorghum hybrid (sorghum *vulgare var*.CSH-5) were from M/S Nath Seeds Pvt. Ltd., Aurangabad, India.

2.2. Collection of Plant Materials

Ten-day-old seedling plants of grain sorghum hybrid (CSH-5) were raised in the laboratory and their leaves were collected as described [19].

2.3. Extraction and Purification of Oxalate Oxidase from Sorghum Leaves

An oxalate oxidase was extracted and purified from sorghum leaves as described [20]. The apparent homogeneity of purified enzyme was revealed in simple PAGE using $AgNO_3$ staining. Insensitivity of purified enzyme towards Cl^- (as NaCl up to 100 mM) and NO_3^- (as $NaNO_3$ up to 8 mM) was confirmed [20].

2.4. Preparation of CA Membrane

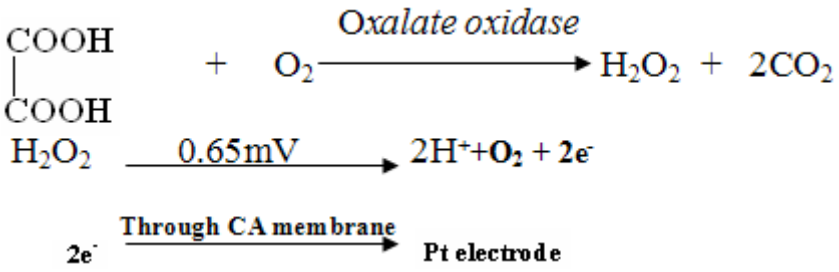
CA cellulose acetate membrane was prepared as described [8] with slight modification. A polymer solution was prepared by dissolving 0.2g of cellulose acetate in 10ml acetone. This polymer solution was poured on a glass petri-dish (diameter 4cm) and then rotated manually on a horizontal flat surface to give an even distribution of polymer solution over glass. The dish was covered with its lid for slow and controlled evaporation of solvent and to create a membrane of even thickness.

2.5. Immobilization of Oxalate Oxidase on CA Membrane

Purified oxalate oxidase was immobilized onto CA membrane as described [8] with modification: Oxalate oxidase (3 mg; 0.85 U/mg) and BSA (50 mg) were dissolved in 250 µl distilled water. 25 µl of enzyme solution and 12.5 µl of 2.5 % (w/v) of glutaraldehyde in distilled water were rapidly mixed and placed on a 4 cm^2 portion of cellulose membrane (Inner membrane). A further 4 cm^2 portion of cellulose acetate membrane (outer membrane) was then placed on enzyme membrane and two glass slides were used to compress both the membrane under mild finger pressure for approximately 5 min. The resulting laminate was then washed with buffer solution (0.05 M sodium succinate buffer, pH 5) to remove excess glutaraldehyde.

2.6. Construction and Response Measurement of Enzyme Electrode

The membrane laminate of oxalate oxidase was mounted over the sensing part of Pt electrode with the help of parafilm. This working (enzyme) electrode was then connected to Ag/AgCl (Reference) electrode through an electrometer (Kethley, Japan, Model: 617) The sensing parts of both the electrodes were immersed into a 15 ml 9.9 ml sodium succinate buffer (0.05M pH 5.0) in a 15 ml beaker kept in a water bath maintained at 37 ^{0}C. The reaction was started by adding 0.1 ml 2 mM oxalate. The enzyme electrode was anodically polarized at +650mV to generate e^- from H_2O_2, liberated from oxalate by immobilized oxalate oxidizer. The electrons thus generated from H_2O_2 were moved to Pt electrode from solution through pores of CA membrane. The electrochemical reactions involved were as follow:



The current in nA was read in electrometer after 2 min of adding oxalate (Fig1). After response measurement, the enzyme electrodes were taken off and washed 4-5 times in 0.05M sodium succinate buffer, pH 5.0 for its use in the next assay.

2.7. Optimization of Working of Enzyme Electrode

2.7.1. Effect of pH

To determine optimal pH for biosensor response, the pH of reaction buffer was varied from pH 2.0 to 8.0 using the following buffer, each at a final concentration of 0.05 M; pH 2.0 to 3.5, sodium citrate; pH 4.0 to 6.0, sodium succinate and pH 6.5 to 8.0, sodium phosphate.

2.7.2. Effect of Temperature

To determine optimum temperature for biosensor response, the reaction mixture was kept in a temperature controlled water bath at different temperature ranging from 20 to 60 °C at a 5 °C interval.

2.7.3. Response Time

To determine the time at which the current was maximum, the current was measured up to 5 min at an interval of 15 second.

2.7.4. Effect of Substrate Concentration

To determine the effect of substrate concentration on electrode response, the concentration of oxalate was varied from 0.02mM to 5.0mM in reaction mixture.

2.8. Preparation of Standard Curve of Oxalate by Enzyme Electrode

The response of enzyme electrode was measured as described above under optimal assay conditions except that oxalate solution of different concentration ranging from 0.02mM to 1.0mM was used. A plot between oxalate concentration and current (nA) was plotted (Fig. 1).

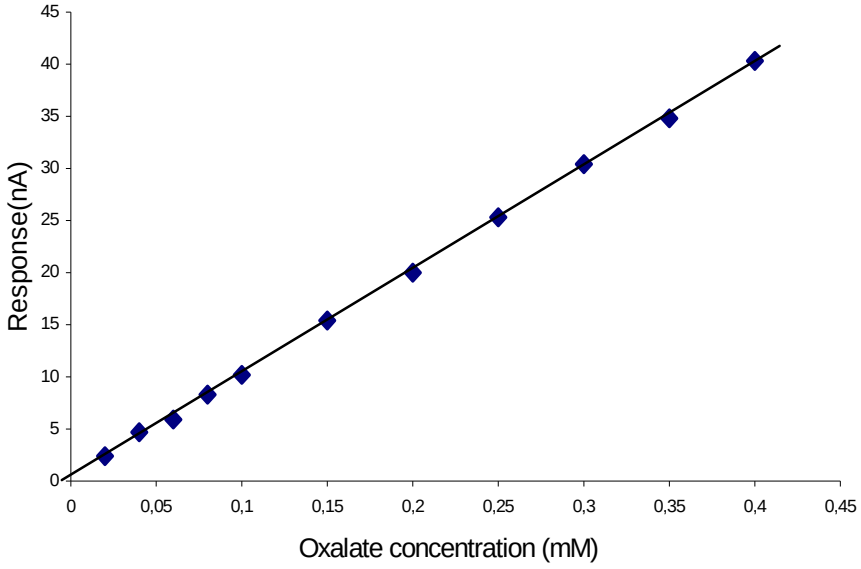


Fig. 1. Standard curve for oxalate by enzyme electrode based on CA membrane bound sorghum leaf oxalate oxidase.

2.9. Effect of Cl^-

To study the effect of Cl^- (as NaCl) on enzyme electrode response in physiological concentration range, aqueous solution of NaCl ranging from 20 mM to 100 mM was diluted by 20 times and its 0.1 ml added to the reaction mixture and volume of reaction buffer was reduced by 0.1 ml. Rest of the procedure was same as described above.

2.10. Effect of NO_3^-

To study the effect of NO_3^-(as $NaNO_3$) on enzyme electrode response in physiological concentration range up to, the 0.1 ml of 20 times diluted aqueous 8 mM $NaNO_3^-$ solution was added to the reaction

mixture and accordingly reaction volume of reaction buffer was reduced by 0.1 ml. Rest of the procedure was same.

2.11. Measurement of Urinary Oxalate by Enzyme Electrode

24 hr urine of apparently healthy individuals of different age and sex group were collected in plastic bottles containing 15 ml conc. HCl. The pH of acidified urine was adjusted to pH 5.5 using HCl or NaOH. 1/20 dilution of urine in reaction buffer and addition of 1mM EDTA to diluted urine was done to achieve excellent electrode response with in linearity range, as suggested [9]. The assay of urinary oxalate was carried out as described for preparation of standard curve of oxalate except that oxalate solution was replaced by pretreated urine. The oxalate content in urine was extrapolated from standard curve between oxalate concentration vs. current (nA) (Fig. 2).The electrode was reused in next assay after ashing it in reaction buffer for 4-5 times.

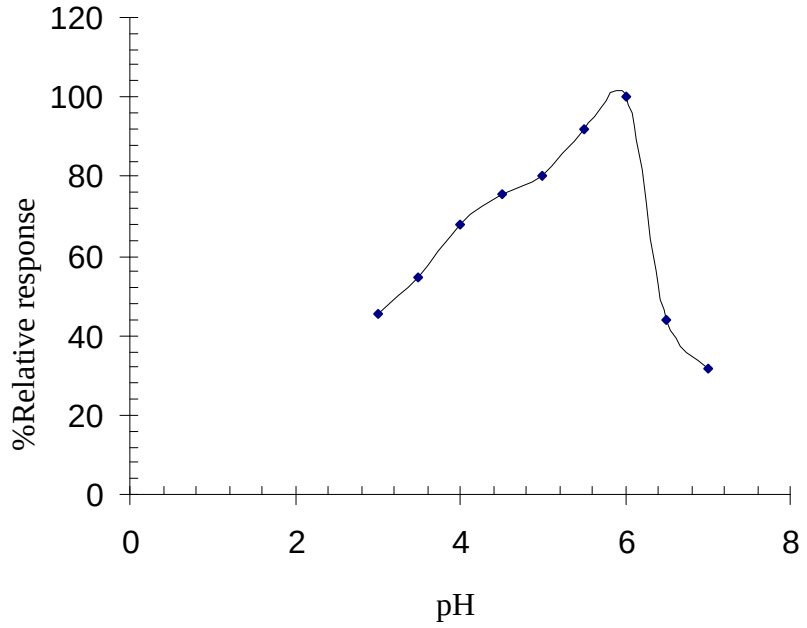


Fig. 2. Effect of pH on CA membrane bound sorghum leaf oxalate oxidase.

2.12. Evaluation of Urinary Oxalate Determination by Enzyme Electrode

The following parameters were studied to evaluate the present method.

2.12.1. Minimum Detection Limit

Minimum detection limit of the method was considered as the concentration of oxalate at which current was 0.4 nA.

2.12.2. Analytical Recovery

To determine the reliability of the method, solid oxalate (10 mg/L, 20 mg/L and 30 mg/L) was added to urine samples and the oxalate content was determined before and after addition of oxalate. The %recovery of added oxalate was calculated.

2.12.3. Precision

To study the reproducibility of the method, the oxalate content was determined in five samples repeatedly (six times) on the same day (within batch) and then in the same sample after their storage at –20 °C for one week (between batch). The within and between day coefficient of variation (CV) was calculated for urinary oxalate determination.

2.12.4. Accuracy

In order to determine the accuracy of the method, the oxalate in urine samples was determined by standard Sigma kit method with modification (x) and by the present method (y).The oxalate values obtained by both the methods was compared and correlated using the regression equation.

2.12.4.1. Modification of Sigma Kit Method for Urinary Oxalate Determination

2.12.4.1.1. Preparation of Barley Oxalate Oxidase

10-day old seedling plants were raised from barley seeds in the laboratory and their leaves were collected as described [19]. Crude oxalate oxidase from leaves of barley seedling plants was homogenized in chilled distilled water in 1:2 ratio in a pestle & mortar. The homogenate was centrifuged at 12000g for 30 min at 4° C and the supernatant was collected. The supernatant was subjected to 0-65% $(NH_4)_2SO_4$ precipitation in cold (4°C) as described by Chiriboga [16] and the pellet was dissolved in 0.05 M sodium succinate buffer pH 3.5 & stored at -20° C until use.

2.12.4.1.2. Determination of Urinary Oxalate by Modified Sigma Kit Method

Pretreatment of urine: One ml 24 hr urine of healthy individual was diluted with 0.1 M sodium phosphate buffer (pH 7.0) containing 0.01 M EDTA in 1:1 ratio and its pH was adjusted between 5.0 to 7.0. Activated charcoal (200mg) was added to diluted urine and mixed by vortex mixer for 5 min and filtered through ‘Whatman’ filter paper No. I.

Assay of urinary oxalate: It was carried out in 15 ml tubes wrapped with carbon paper. To 0.1 ml of oxalate reagent A, 0.5 ml of pretreated urine and 1.0 ml of oxalate reagent B were added and mixed by vortex mixer. After incubation at 37°C for 5 min, A_{590} was read. The oxalate concentration was determined from standard curve prepared between oxalate concentrations ranging from 0.1 mM to 1.0 mM Vs $A_{590.}$

Oxalate reagent A: It was prepared by mixing of 3.2 mmoles of 3-(dimethylamino) benzoic acid (DMAB) and 0.22 mmoles of 3-methyl-2-benzothiozolinone hydroazone (MBTH) in one litre of 0.05 M sodium succinate buffer, pH 3.5. It was stored in amber coloured bottle at 4 °C until use.

Oxalate reagent B: It was prepared by mixing of 1mg of partial purified barley oxalate oxidase and 1mg horse radish peroxidase (RZ = 1.0) in 10ml of deionized water. It was stored in amber coloured bottle at 4°C until use.

2.16. Storage and Stability of Enzyme Electrode

The enzyme electrode was stored in0.05 M sodium succinate buffer (pH 5.5) at 4 °C when not in use. The activity of electrode was tested everyday at 35 °C in a temperature controlled water bath

3. Results and Discussion

A Cl^- and NO_3^- insensitive oxalate oxidase purified from leaves of 10-day-old seedling plants of grain sorghum hybrid (CSH-14) was cross linked with BSA through glutaraldehyde and then adsorbed in between two CA membranes. A procedure is described for construction of an oxalate biosensor (enzyme electrode) employing this CA membrane bound sorghum leaf enzyme. However, this enzyme electrode is better than those based on barley seedling enzyme, as it is unaffected by Cl^- and NO_3^- normally found in biological fluids. The optimal working condition of this electrode was as follows:

3.1. Optimization of Biosensor

3.1.1. Optimum pH

A clear optimal response i.e. activity of CA membrane bound oxalate oxidase was seen at pH 6.0 (Fig. 2), which is higher than that of optimum pH of free enzyme (pH-5.0) (Table 1) [20]. An increase in pH optima of oxalate oxidase after immobilization has been reported for barley enzyme on nylon tubing [21], polyamide membrane [3], PB film [13]**,** polypyrrole hybrid film [15] and alkylamine glass beads [22], beet stem enzyme coupled to polyethylene glycol [23] and sorghum leaf enzyme coupled to alkylamine and arylamine glass beads [24], PVA membrane [25] and PVC sheet [26],. The increase in optimum pH of sorghum enzyme after immobilization on CA membrane could be due to loss of $–NH_2$ group(s) on the surface of enzyme as result of glutaraldehyde cross linking between BSA and enzyme.

3.1.2. Optimum Temperature and Thermal Stability

The enzyme showed a slight decrease in optimum temperature from 37 °C to 35 °C after immobilization on CA membrane (Fig. 3, Table 1)). Earlier, a decrease in optimum temperature for maximum activity has been reported for barley oxalate oxidase after immobilization on pig intestine membrane (26 °C) [4], gelatin (26 °C) [5], alkylamine glass beads (30 °C) [22] , alkylamine glass beads affixed inside a glass beaker [27] and Amaranthus leaf enzyme on alkylamine glass beads[24] but no change in optimum temp after immobilization of sorghum leaf enzyme onto PVC sheet [26], The enzyme showed a decrease in thermostability after immobilization. The CA membrane bound enzyme retained 50 % activity ,when heated at 50 °C for 15 min and no activity when heated at 80 °C for 15 min. Earlier a decrease in thermostability was found for barley enzyme after immobilization on to alkylamine glass beads [22] and Amaranthus leaf enzyme immobilized onto alylamine glass beads [28]. The decrease in thermal stabilityion of strain in the enzyme after glutaraldehyde crosslinking.

3.1.3. Response Time

The response time of the enzyme electrode was about 2 min (Table 1), which is lower than that of oxalate biosensor based on CA membrane bound barley enzyme [8] Sorghum leaf enzyme on PVC sheet (15 min) [26], PVA membrane (10min) [25] but higher than that for carbon paste electrode employing barley oxalate oxidase and horseradish peroxidase (0.5 sec) [11].

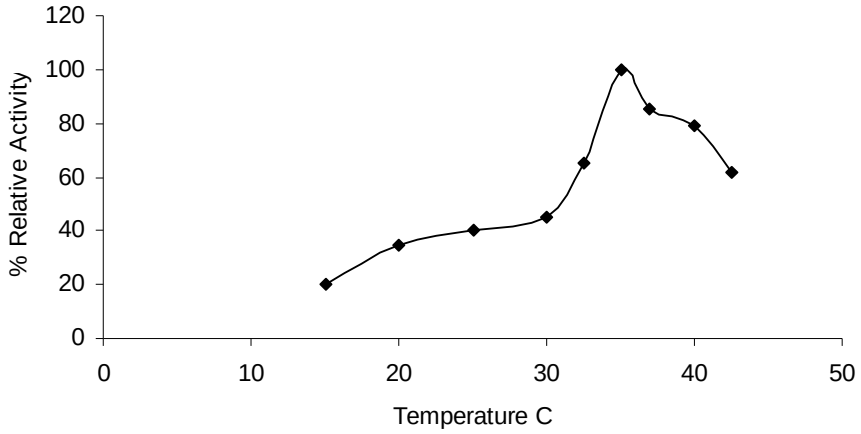


Fig. 3. Effect of incubation temperature on CA membrane bound sorghum leaf oxalate oxidase.

Table 1. A comparison of kinetic properties of free and cellulose acetate membrane bound grain sorghum leaf oxalate oxidase.

Parameter	**Free Membrane bound**	
Optimum pH	5.0	6.0
Temperature for maximum activity (°C)	40	35
Time for linearity/ response time (min)	2	2
Km for oxalate (mM)	0.08	0.33

3.1.4. Determination of K_m

There was a hyperbolic relationship between oxalate concentration and response of enzyme electrode up to 2 mM. K_m value for oxalate as calculated from Lineweaver-Burk plot was 0.33 mM (Fig 4), which is higher than that reported for free enzyme (0.062 mM) (Table 1) [20].An increase in Km value of sorghum enzyme after immobilization on alkyl and arylamine glass beads [24] PVA membrane [25], PVC sheet [26], CAmembrane [8] and polypyrrole hybrid film [15] have been reported. The increase in K_m of enzyme after immobilization indicates its decreased affinity towards its substrate. The change in Km might be due to change in its kinetic pattern after immobilization. The kinetics of immobilized enzyme is affected by a number of factors such as (i) conformational change due to chemical modification of enzyme, (ii) steric effects which occur because often a proportion of enzyme molecules is immobilized in a position relative to the support surface in such a way that the active site of enzyme is relatively inaccessible to substrate molecule, (iii) partitioning effects which may arise from electrostatic or hydrophobic interaction between matrix and low molecular weight species present in solution, leading to a modified microenvironment, (iv) mass transfer or diffusional effects arising from diffusional resistance to the substrate from bulk solution to the active site and from the diffusion of products of reaction back to bulk solution.

3.2. Effect of Cl

The CA-membrane bound enzyme was unaffected by Cl^- (as NaCl) up to 100mM, similar to grain sorghum leaf enzyme bound to alkylamine glass beads [29], Amaranthus leaf enzyme bound to alkyl and arylamine glass beads [30]. Earlier , a strong inhibition of PEG linked beet stem enzyme by Cl^- at 10 mM [23] and nylon tube bound barley enzyme by Cl^- when used in a continous flow system[17] alkylamine and arylamine glass bound barley enzyme by Cl^- at 1.0mM concentration [22] have been reported.

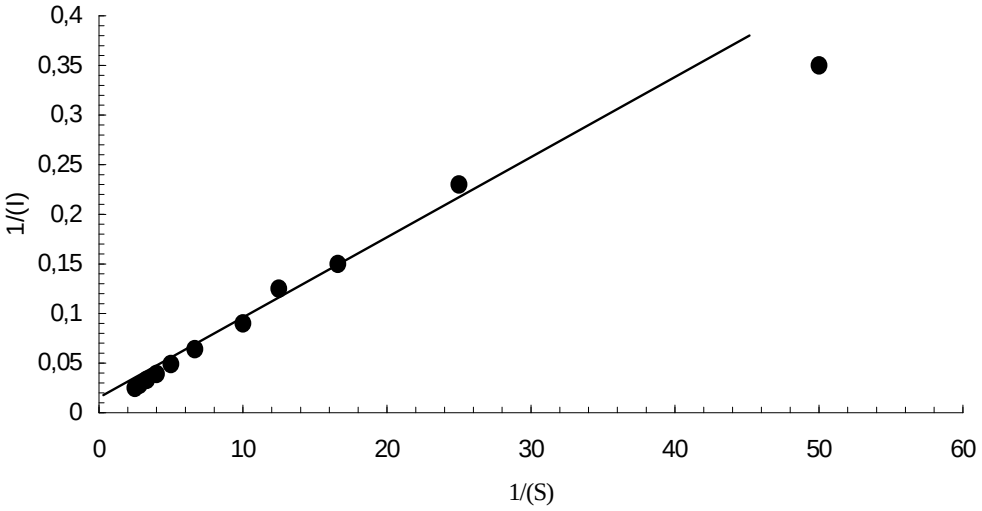


Fig. 4. Lineweaver-Burk plot for CA membrane bound sorghum leaf oxalate oxidase.

3.3. Effect of NO_3^-

NO_3^- (as $NaNO_3$) up to 8 mM (physiological conc.) had practically no effect on CAmembrane bound sorghum leaf oxalate oxidase, Earlier, barley and beet stem enzymes were found to be inhibited by NO_3^- in the concentration range 0.01 mM to 1 mM [18,23]. These results indicated the better suitability of grain sorghum leaf oxalate oxidase over barley and beet stem enzymes for construction of an enzyme electrode for determination of oxalate in biological fluids, which normally contain Cl^- and NO_3. $^-$A method for amperometric determination of urinary oxalate was developed employing this enzyme electrode. The method is based on generation of e^- from H_2O_2 produced by oxidation of oxalate by CA membrane bound oxalate oxidase. The flow of e^- i.e. current in nA was measured by electrometer, which is directly proportional to oxalate conc. The method has the advantage that it not only provides the rapid measurement of oxalate but also unaffected by Cl^- and NO_3^- normally found in biological fluids. There was a linearity between oxalate concentration ranging from .02 mM to 0.4 mM and current (nA) (Fig. 2).

3.4. Evaluation of Method of Oxalate Determination

3.4.1. Minimum Detection Limit

The minimum detection limit of the present method was 0.02mM, which is similar to that by PVC membrane bound barley enzyme electrode (0.02 mM) [9] and biosensor based on barley oxalate oxidase and SIRE technology (0.02 mM) [12] but lower than oxalic acid sensor using a bio-thermochip (0.2 mM) [30] and carbon paste electrode containing barley oxalate oxidase and horseradish peroxidase (0.09 mM) [11].

3.4.2. Recovery Studies

The analytical recovery of added oxalate in urine samples (10mg/l, 20 mg/l.30 mg/l) as measured by the enzyme electrode was 92.56 %, 98.83 % and 96.38 % respectively (Table 2), which is comparable with those of employing biosensor barley oxalate oxidase immobilized on pig intestine membrane (93.5 to 106.4 %) [4] and gelatin (98 %, 96 % recovery for 17.5 mg/l and 35.0 mg/l oxalate added

respectively) [4] but higher than methods such as continuous flow method using oxalate oxidase bound to nylon tubing (95.9 %) [17] discrete method using banana oxalate oxidase immobilized to acrylamine membrane (86-96 %) [31].

Table 2. Analytical recovery of added oxalate in urine measured by enzyme electrode based on cellulose acetate membrane bound Sorghum leaf oxalate oxidase.

Oxalic added mg/L	Oxalate found (mean) (n=7)	% Recovery
Nil	14.2	-
10	22.4	92.56
20	33.8	98.83
30	42.6	96.38

3.4.3. Precision

This study was carried out to assess the reproducibility and reliability of the method. Within and between assay coefficients of variation for urinary oxalate determination by the enzyme electrodes were 3.09 % and 6.5 % respectively (Table 3), which is quite close to those reported by the other methods using alkylamine bound sorghum oxalate oxidase (<3.5 % and <6.46 %) [29], oxalate oxidase immobilized to pig intestine membrane on the tip of oxygen electrode (CV 3.2 %, 4.2 % and 5.6 %) [3] but higher than that employing to spinach tissue homogenate immobilized onto Teflon membrane of DO probe (1.8 %) [6] These results reveal the high reproducibility and reliability of present method.

Table 3. Within and between assay coefficients of variation (CV) for determination of urinary oxalate by enzyme electrode based on cellulose acetate membrane bound Sorghum leaf oxalate oxidase.

Oxalic acid (mg/24h)	Mean	% CV
Within assay 26.8 24.3 25.5 25.5 25.5	25.52	3.1
Between assay 25.2 26.4 23.8 22.4 22.4	24.04	6.5

3.4.4. Accuracy

To test the accuracy of the method, the urinary oxalate values obtained by the present method (y), were compared with those obtained by modified enzymic colorimetric method of Sigma(x). The urinary oxalate value by both methods were almost similar and the correlation coefficient (r) was 0.99, indicating high accuracy of the method.

3.5. Reusability and Storage

The enzyme electrode lost 50 % of its initial activity after its 100 uses for over 30 days, when stored in 0.05 M sodium succinate buffer pH 6.0 at 4 °C.

3.4 3.6. Interference Study

Interference by metal ions found in urine poses a potential problem in determination of oxalate by an enzyme electrode. Na^{+}, K^{+}, Mn^{2+} and Mg^{2+} (as their chloride and nitrate salt) had no interference, as there was not any noticeable change in relative response. However Zn^{2+}, Mn^{2+}, Mg^{2+}, Ca, $^{2+}$ Fe^{2+}, (as sulfate salts) and Mn^{2+}, Mg^{2+} (as Cl^{-} salt) caused slight decrease in electrode response (Table 4). To overcome this effect EDTA (1mM) was added, which reversed these effects. EDTA has higher affinity for metal ions at an ambient concentration (1mM), which could sequester the metal ions [9].

Table 4. Effects of various metal ions on the relative response of enzyme electrode based on cellulose acetate membrane bound Sorghum leaf oxalate oxidase.

Compound added (mM)	Relative response	Response after addition of EDTA (1mM)
None	100	-
KCl	100	100
NaCl	100	100
$MnCl_2$	54.4	100
$CuCl_2$	57.8	100
$MgSO_4$	60.1	82.4
$CaSO_4$	78.8	88.6
Fe_2SO_4	41.61	80.7
$ZnSO_4$	47.79	100
$CuSO_4$	52.6	92.0
$NaNO_3$	100	100

3.7. Determination of Oxalate

3.7.1. Determination of Urinary Oxalate

Oxalate value in 24h urine from apparently healthy male and female in three age groups i.e. children (1-20 yr) , young (21-45 yer) & old persons (> 45yr) (n=20 in each group) and urinary stone formers in same groups were measured by the present enzyme electrode. The results are given in Table 5, which show that the urinary oxalate value was in the range, 7.2 mg/L to 32.4 mg/L and 9.4 mg/L to 38.4 mg/L with a mean of 19.01 mg/L and 20.1 mg/L in healthy male and female respectively . These values are comparable to those reported earlier such as 14.0 to 35.6 mg/L by continuous flow system using barley oxalate oxidase immobilized to nylon tubing [17], 4.8 t to7.9 mg/l by immune complex of banana oxalate oxidase [32], 6.6-35.4 mg/L urine by acrylamide membrane bound oxalate oxidase [31] 9.0 to 28.0 mg/L with a mean of 18.16 mg/L with alkylamine glass bound Amaranthus leaf oxalate oxidase [30] The urinary oxalate value in stone formers (male and female) was in the range 22.4 mg/L to 54.8 mg/L and 20.6 mg/L to 52.6 mg/L with a mean of 37.57 mg/L and 35.9 mg/L male and female respectively, which is 2-3 times higher than those in healthy individuals, similar to earlier reports [32].

Table 5. Urinary oxalate value in healthy individuals and stone formers as determined by enzyme electrode based on cellulose acetate membrane bound Sorghum leaf oxalate oxidase. The values are mean ± SD (n=20) for each group.

Age group (years)	**Sex**	**Oxalate value (mg/24 hr) (mean ±SE)**	
		Healthy individuals	**Stone formers**
Children (1-20)	Male Female	12.45±5.7 14.9±2.3	29.96±2.0 31.53±1.8
Adult (21-45)	Male Female	25.57±3.03 16.74±4.9	43.96±2.1 41.43±2.3
Old (46 and above)	Male Female	24.03±2.8 28.38±3.2	38.79±2.4 34.76±2.5

Mean: 19.01 Healthy male 37.57 Stone former male
20.10 Healthy female 35.91 Stone former female

5. Conclusion

An electrochemical oxalate biosensor was constructed by mounting CA membrane bound grain sorghum leaf oxalate oxidase to Pt electrode. The biosensor measures current in nA which is directly proportional to oxalate concentration. The electrode has the advantage over other electrodes employing barley enzyme that it does not require the pretreatment of biological samples for removal of Cl^- and NO_3^-.

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Guide for Contributors

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Sensors & Transducers Journal (ISSN 1726-5479) provides an advanced forum for the science and technology of physical, chemical sensors and biosensors. It publishes state-of-the-art reviews, regular research and application specific papers, short notes, letters to Editor and sensors related books reviews as well as academic, practical and commercial information of interest to its readership. Because it is an open access, peer review international journal, papers rapidly published in *Sensors & Transducers Journal* will receive a very high publicity. The journal is published monthly as twelve issues per annual by International Frequency Association (IFSA). In additional, some special sponsored and conference issues published annually. *Sensors & Transducers Journal* is indexed and abstracted very quickly by Chemical Abstracts, IndexCopernicus Journals Master List, Open J-Gate, Google Scholar, etc.

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