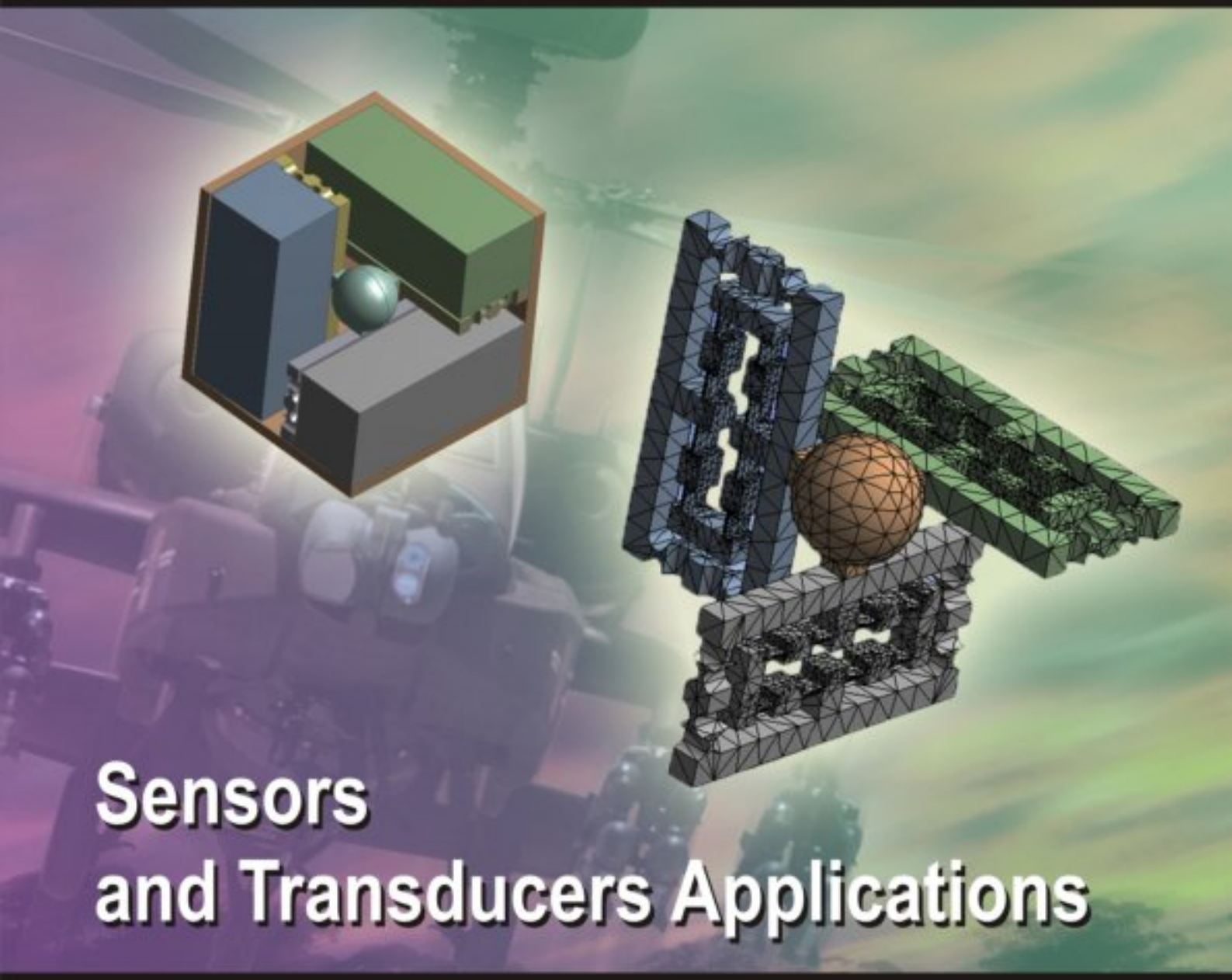
ISSN 1726-5479

SENSORS & TRANSDUCERS

8 vol. 119 /10



Sensors and Transducers Applications

International Frequency Sensor Association Publishing

IFSA

Sensors & Transducers

Volume 119, Issue 8, August 2010 **www.sensorsportal.com** **ISSN 1726-5479**

Sensors & Transducers Journal (ISSN 1726-5479) is a peer review international journal published monthly online by International Frequency Sensor Association (IFSA).
Available in electronic and on CD.

Sensors & Transducers Journal

Contents

Volume 119
Issue 8
August 2010

www.sensorsportal.com

ISSN 1726-5479

Research Articles

Authors are encouraged to submit article in MS Word (doc) and Acrobat (pdf) formats by e-mail: editor@sensorsportal.com
Please visit journal's webpage with preparation instructions: http://www.sensorsportal.com/HTML/DIGEST/Submition.htm

International Frequency Sensor Association (IFSA).

The Second International Conference
on Sensor Device Technologies and Applications

SENSORDEVICES 2011

August 21-27, 2011 - French Riviera, France

IFSA
www.sensorsportal.com

Important deadlines:

Submission deadline	March 23, 2011
Notification	April 30, 2011
Registration	May 15, 2011
Camera ready	May 22, 2011

Tracks:
- Sensor devices
- Photonics
- Infrared
- Ultrasonic and Piezosensors
- Sensor device technologies
- Sensors signal conditioning and interfacing circuits
- Medical devices and sensors applications
- Sensors domain-oriented devices, technologies, and applications
- Sensor-based localization and tracking technologies

http://www.iaria.org/conferences2011/SENSORDEVICES11.html

The Fifth International Conference on Sensor
Technologies and Applications

SENSORCOMM 2011

August 21-27, 2011 - French Riviera, France

IFSA
www.sensorsportal.com

Important deadlines:

Submission deadline	March 23, 2011
Notification	April 30, 2011
Registration	May 15, 2011
Camera ready	May 22, 2011

Tracks:
- APASN: Architectures, protocols and algorithms of sensor networks
- MECSN: Energy, management and control of sensor networks
- RASQOFT: Resource allocation, services, QoS and fault tolerance in sensor networks
- PESMOSN: Performance, simulation and modelling of sensor networks
- SEMOSN: Security and monitoring of sensor networks
- SECSED: Sensor circuits and sensor devices
- RIWISN: Radio issues in wireless sensor networks
- SAPSN: Software, applications and programming of sensor networks
- DAIPSN: Data allocation and information in sensor networks
- DISN: Deployments and implementations of sensor networks
- UNWAT: Under water sensors and systems
- ENOPT: Energy optimization in wireless sensor networks

http://www.iaria.org/conferences2011/SENSORCOMM11.html

The Fourth International Conference on Advances
in Circuits, Electronics and Micro-electronics

CENICS 2011

August 21-27, 2011 - French Riviera, France

IFSA
www.sensorsportal.com

Important deadlines:

Submission deadline	March 23, 2011
Notification	April 30, 2011
Registration	May 15, 2011
Camera ready	May 22, 2011

Tracks:
- Semiconductors and applications
- Design, models and languages
- Signal processing circuits
- Arithmetic computational circuits
- Microelectronics
- Electronics technologies
- Special circuits
- Consumer electronics
- Application-oriented electronics

http://www.iaria.org/conferences2011/CENICS11.html

Sensors & Transducers Journal, Vol. 119, Issue 8, August 2010, pp. 129-141

Sensors & Transducers

ISSN 1726-5479

http://www.sensorsportal.com

Amperometric Glucose Biosensor Based on Immobilization of Glucose Oxidase in Polyethylenemine and Poly (carbamolylsuphonate) Polymer Matrix

[a]U. B. Trivedi, [a]D. Lakshminarayana, [b]I. L. Kothari, [c]N. G. Patel, [d]H. N. Kapse, [a]P. B. Patel, [e*]C. J. Panchal

[a]Department of Electronics, [b]Department of Bio-Sciences, Sardar Patel University, Vallabh Vidyanagar-388120, Gujarat, India

[c]Department of Chemistry and Physics, University of North Florida, Jacksonville, FL, USA

[d]Institute of Science and Technology for Advanced Studies and Research (ISTAR), Vallabh Vidyanagar-388120, Gujarat, India

[e]Applied Physics Department, Faculty of Technology and Engineering, M. S. University of Baroda, Vadodara-390001, Gujarat, India

[*]Tel.: +91 9825094761, fax: +91 265 2423898

E-mail: cjpanchal_msu@yahoo.com

Received: 19 June 2010 /Accepted: 17 August 2010 /Published: 31 August 2010

Abstract: A low cost, easy to fabricate, simple to operate, portable and disposable biosensor based upon the enzyme glucose oxidase (GOD) has been developed. A polymer matrix of Polyethylenemine (PEI) and Poly Carbamoylsulphonate (PCS) hydrogel has been used for the immobilization of GOD on a Platinum (Pt) tip of a screen printed graphite electrode. A multi channel potentiostat has been utilized in the electrode configuration for the amperometric measurement of glucose. The developed Pt/PCS+PEI/GOD glucose biosensor system could offer a reliable method of glucose determination in standard stock solutions. The designed glucose bio-sensor system has been duly characterized and the results obtained are presented. The biosensor has been found to show a sensitivity of 0.72 ± 0.15 nA/μM. Also, the specificity of the enzyme electrode and the storage stability of the GOD based biosensor system have been studied. The sensor system has also been tested with the real samples of fruit juice, soft drink and human blood. The biosensor system has been found to possess a potential for food analysis and clinical utility. *Copyright © 2010 IFSA.*

Keywords: Biosensor, Amperometry, Glucose, Glucose oxidase, Polymer matrix.

1. Introduction

Improvevbbbbment of "life quality "is one of the most important objectives of global research efforts dealing with any area of Science and Technology. The quality of life is closely linked to the detection and control of diseases, food quality and safety, and quality of environment. Glucose ($C_6H_{12}O_6$) is a major component of animal and plant carbohydrates and is the primary source of energy. In medicine and animal physiology, the term 'blood sugar' refers to the concentration of glucose in the blood. Diabetes mellitus is a chronic lifelong disease caused by a carbohydrate metabolic block, in which the human body does not produce or properly use insulin, a hormone needed to convert sugars, starches and other foods in to energy. Long-term effects include nephropathy and kidney disease, blindness, nerve damage which often leads to lower limb amputations, heart disease and stroke. In order to maintain normal blood sugar levels, the diabetic patients need regular sugar level monitoring and also need daily intake of painful insulin injections. For non diabetics too, a dire necessity has arisen for undergoing regular checkups for sugar level detection. The sugar level detection and control at an early stage may help prevent or delay the complications of the diabetes. Hence, a great deal of research effort is being devoted towards developing a continuous, fast and sensitive blood glucose level monitoring system.

A quantitative determination of glucose has, therefore, become an important aspect in biochemistry, clinical chemistry and food analysis. Numerous methods like spectrophotometry, high performance liquid chromatography (HPLC), polarimetry, capillary electrophoreses etc. have been widely reported [1-10] for the determination of glucose concentration. However, most of these methods are lengthy and/or expensive in nature. Also, these methods are not found to be very appropriate for rapid field tests. In view of the increasing concern over the quantitative determination of glucose (for medical as well as non-medical reasons), a need has been arising, of late, for developing rapid, portable and low cost alternate methods for the glucose analysis. Biosensors have been emerging as potential and alternate methods of food and clinical analysis. Indeed, several researchers reported the fabrication of a variety of amperometric type glucose biosensors for the detection of glucose concentration [11-18]. The amperometric biosensor based on enzyme electrodes has been the primary choice of the biosensing systems because of its high sensitivity, short response time and low cost of instrumentation [19-20]. In the construction of an amperometric biosensor, a number of immobilization techniques such as physical entrapment, chemical immobilization in an inert matrix, covalent attachment to electrode surfaces, cross linking etc. have been used to immobilize the relevant enzyme [21-22]. On the other hand, conducting or non conducting polymer films prepared by an electrochemical polymerization of a relevant monomer have also been successfully used as enzyme immobilization media [23-24]. The electropolymerized polymer films of polypyrrole (PPy) and poly o-phenylenediamine (PPD) received considerable attention in the preparation of glucose biosensor [18, 25-28]. However, the aspects of polymer film structure, perm selectivity character, mechanism of enzyme incorporation etc. remained uncertain. In order to improve the performance of the glucose biosensor by enhancing the electron transfer and minimizing the electro chemical interference, various researchers reported the usage of nano particles [29-31] and carbon nano tubes (CNTs) [32-34]. Despite its advantages, the barrier of dispersing CNTs is known to affect the immobilization of the enzyme. Recently, Hong Wu et al [35] reported a novel but a complex as well as an expensive type of functionalized graphene sheet (FGS) based biosensor for sensitive glucose sensing. An important aspect of the whole gamut of enzyme based glucose biosensor research work appears to find the most effective immobilization method with minimum electro chemical interference.

It is, therefore, not surprising that a thorough survey and study of the available literature on the fabrication of glucose biosensors, starting from the first generation Clark type biosensors [36] up to the most recent carbon nano tube based ones, indicate that there is still a scope and need for the development of an enzyme based glucose biosensor which should be simple to construct and easy to

operate. The authors have recently reported [37, 38] the fabrication of amperometric fructose and potentiometric urea biosensors utilizing a unique polymer matrix of polyethylenemine (PEI) and poly carbamolylsuphonate (PCS) hydrogel for the immobilization of the enzymes. In both the cases, the authors have found the entrapment of the enzymes in the PCS+PEI matrix very attractive due to a strong adhesion between the enzyme and matrix. The enzyme activity could be retained for longer duration with high sensitivity and low electro chemical interference. It is thus natural for the authors to study the efficacy of the PCS+PEI matrix in the development of a glucose biosensor. The authors, therefore, report the development of a glucose oxidase (GOD) enzyme based amperometric glucose biosensor utilizing the, (PCS+PEI) polymer matrix in this communication.

2. Experimental

2.1. Chemicals and Reagents

The various solutions utilized in the present investigation were all prepared with analytical reagent grade chemicals and double distilled water. The various enzymes, monomers, polymers, pastes and the main chemicals used in this investigation are listed below with their makes.

2.1.2. Enzyme and Polymers

- Glucose oxidase (GOD) (EC: 1.1.34, 50,000 Unit, G-6641) (Sigma, St. Louis, USA)
- Polyethylenemine (50 % w/v aqueous solution) (PEI) (Sigma, USA)
- Poly (carbamoylsulphonate) (PCS) hydrogel (Sens Lab, Germany)

2.1.3. Pastes for Screen Printed Fabrication of the Electrodes

- Platinum paste R-474 (DPM-80) (Ercon, USA)
- Graphite paste G-449 (Ercon, USA)
- Silver/Silver chloride paste R-414 (DPM-68) (Ag/AgCl) (Ercon, USA)

2.1.4. Chemicals

- Disodium hydrogen phosphate 67 mM (Merck, Darmstadt, Germany)
- Sodium dihydrogen phosphate 67 mM (Merck, Darmstadt, Germany)
- Potassium chloride 0.1 M KCl, (Merck, Darmstadt, Germany)
- Sodium chloride (Merck, Darmstadt, Germany)
- Sodium hydroxide (Merck, Darmstadt, Germany)
- D (+) glucose (Sigma, Deisenhofer, Germany)
- Ascorbic acid (Sigma, St. Louis, USA)

2.1.5. Apparatus

- Micro pipette (Eppendorf, Germany)
- Multi-Channel ISE/pH/mV/ORP/Temperature Bench top Meter (Thermo Orion, USA)
- Ag/AgCl reference electrode (Thermo Orion, USA)

2.2. Electrode Fabrication and Enzyme Immobilization

The most common type of working electrodes in amperometric biosensors are platinum, gold and graphite for the anodic oxidation. In the present investigation, platinum (Pt) was chosen due to its high chemical inertness and also it provides a wide range of anode working potentials with low electrical resistivity. The electrode fabrication procedure for the glucose biosensor was essentially the same as that reported, earlier, by the authors [37] for the amperometric fructose biosensor. However, for the purpose of continuity and clarity, some of the details of the electrode fabrication are being repeated here.

The base transducer used in the present work was also a screen printed graphite electrode on a Melinex sheet of 150 μ thicknesses. The graphite paste was screen printed using screens having a mesh size of 68 μ. Before and after screen printing, the sheet was annealed for 60 minutes at 373 K. After that, using a laminating machine (SANON, CR-309, India) a round shaped platinum disk having a 3 mm diameter was sandwiched between one end of the graphite screen printed electrode and a blank polyester sheet having a punched hole for exposing the platinum tip. Hence, the lamination was done in such a way, leaving a round shaped platinum tip of about 0.3 cm diameter exposed for the enzyme immobilization purpose later. The other end of the electrode was left un-laminated for the electrical contact purpose. The entire lamination process was carried out at 413 K. A large number of graphite based platinum tipped electrodes were fabricated in a similar way.

A PCS solution was prepared by dissolving 150 mg PCS prepolymer in 400 μl deionized water. Next, a PEI solution was prepared by dissolving 2.5 mg PEI prepolymer in 50 μl deionized water. Then, the GOD enzyme of 4.18 mg was mixed with a 100 μl potassium phosphate buffer solution. The enzyme solutions were stored at -20°C and were used later as the enzyme stock solutions. A mixture of PCS and PEI solution was prepared by slowly adding PEI solution using a micro pipette (Eppendorf, Germany) in to the acidic PCS solution. The PEI (2.5 % in aqueous solution) was added till the pH value of the mixture solution reached 5.4. Then, 50 μl of GOD stock solution was added to the mixed solution of PCS and PEI in a proportion of 1:1 and gently stirred at the room temperature of 303 K. Using the micro pipette, 2 μl mixture of GOD and PCS+PEI solution was dropped on the platinum tip of the working electrode at the room temperature of 303 K. A batch of several working electrodes were prepared accordingly and were all kept at 4 ºC over night. Fig. 1 shows a schematic of a typical working electrode for immobilization of GOD through PCS+PEI polymer matrix for use in the amperometric measurements.

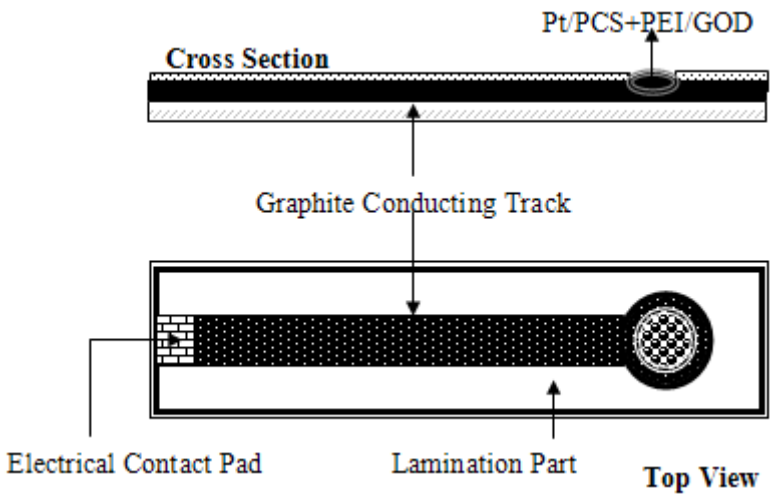


Fig. 1. Schematic of a typical working electrode of Pt/PCS+PEI/GOD for amperometric measurements.

2.3. Measurements

The glucose, in the presence of GOD, produces gluconic acid and hydrogen peroxide (H_2O_2). By applying a suitable potential of, say +600 mV, to the platinum electrode (against Ag/AgCl electrode) an electrochemical oxidation of H_2O_2 takes place. The electrochemical reactions are as follows:

$$\text{Glucose} + O_2 \xrightarrow{\text{GOD}} \text{Gluconic acid} + H_2O_2$$

$$H_2O_2 + \xrightarrow{0.6\ \text{V}} O_2 + 2H^+ + 2e^-$$

The electron current thus generated is an indicative of the glucose concentration. A calibration curve can then be obtained and used for detecting the unknown glucose concentrations.

In the present investigation, a Multi-Channel ISE/pH/mV/ORP/Temperature Bench top Meter (Thermo Orion, USA) was used in the electrode configuration for the amperometric measurements of glucose detection. The fabricated enzyme based glucose biosensors were used as the working electrodes with Ag/AgCl as the reference electrode. The electrodes were suitably dipped in to 30 ml of potassium phosphate buffer solution and connected to the potentiostat. At the working electrode, a potential of +600 mV with reference to the Ag/AgCl reference electrodes was applied for the glucose analyte determination. However, before starting the actual glucose measurements with the fabricated biosensors, a pre conditioning step was carried out by applying a potential of +1.2 V at the working electrode (against Ag/AgCl) for about 270 sec at room temperature of 303 K. This pre conditioning step was carried out for improving the surface morphology of the electrodes.

A 1 M glucose stock solution was prepared first. The glucose sensor was investigated in a phosphate buffer (pH 6.8) of 30 ml solution in a measuring cell with the addition of 10 ml/1 M glucose stock solution. Then, 30 µl of the stock solution was gradually and manually added in to this buffer solution by a micro pipette (Eppendorf, Germany) along with a continuous stirring for about 10 to 12 minutes to get the stable base line. A stabilized (after about 15-18 sec.) reading of the amperometric response was then recorded on the potentiostat against the known glucose concentration. Then, solutions of the known glucose concentrations were carefully added, as the test samples, to the above buffer to study the glucose testing ability of the sensor. The procedure was repeated for various other concentrations of glucose in steps of 30 µl and a calibration curve was, thus, obtained. It is to be noted here that the sensor was connected to the potentiostat continuously during the entire calibration procedure.

Commercial brands of fruit juice (orange and apple juices), soft drinks (fanta and cola) and also, human blood samples collected from the local pathological testing laboratories where used as the *real sample*s for the detection of their glucose concentration levels by the fabricated Pt/PCS+PEI/GOD glucose biosensor system. The results obtained were than compared with those obtained by the commercial methods.

3. Results and Discussion

3.1. Response Behaviour

The effect of the working potential on the amperometric response of the enzyme electrode was examined in the potential range of 300–900 mV against Ag/AgCl reference. The highest amperometric response was obtained at a potential of 600 mV (Fig. 2).

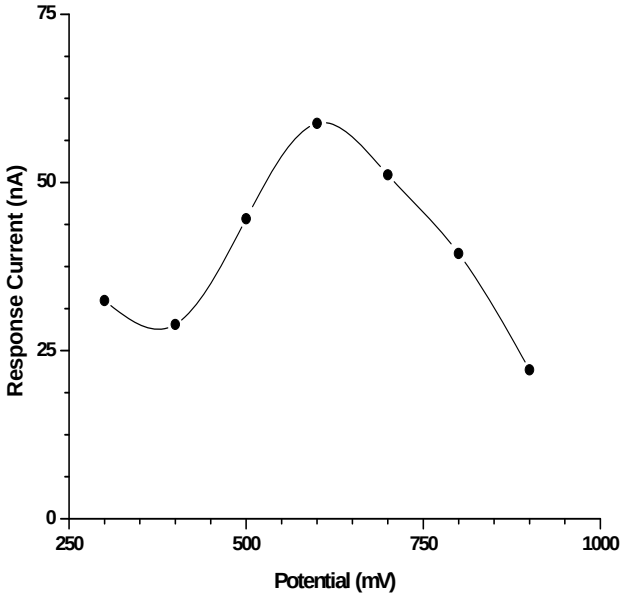


Fig. 2. Effect of working potential on the response.

Fig. 3 shows the variations of the amperometric current generated in the bio-electro chemical cell when standard glucose stock solution samples of 20 µg/ml were added in steps. After each addition, the current was found to increase proportionately. The average increase was 15 ± 0.50 nA per each 20 µg/ml. The response was found to be very fast (10 to 15 sec) and the signal was stable between the sample additions. The recovery time was about 30 seconds. The response time depends upon the analyte, co-substrate and the product transport through different membranes. The thickness and permeability of the polymer matrix (membrane) are very critical in determining the response behaviour. They also depend upon the activity of the molecular reorganization system. The higher the activity of the system, the smaller will be the response time.

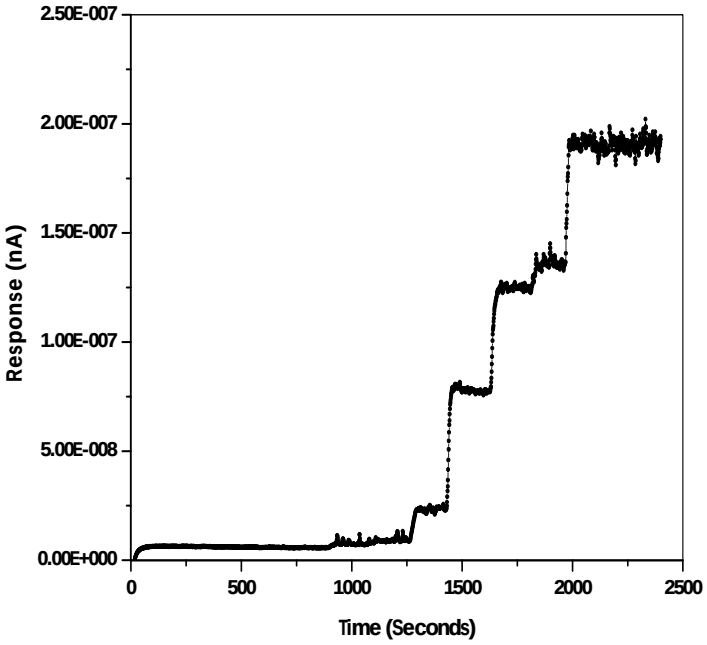


Fig. 3. The amperometric response of the enzyme electrode to successive glucose additions.

3.2. Calibration Curve and Sensitivity

Using the amperometric responses obtained from Fig. 3, a typical calibration curve for the developed glucose biosensor was obtained as shown in Fig. 4. It is clearly seen from the Fig. 4 that the enzyme electrode produces a linear steady state amperometric response up to 10-12 mM glucose. The calibration curve exhibits a good linearity with a correlation coefficient of 0.9998 (n=10).

The existence of this linear relationship between the current and concentration of glucose is important for the accurate determination of glucose levels in human blood which lie within a narrow range of 3.5 to 5.0 mM [39]. The response current approached a saturation value at higher glucose concentrations in the present case and the trend was found to be similar for a number of electrodes. The sensitivity or slope of the linear calibration curve of the Pt/PCS+PEI/GOD glucose biosensor system has been found to be 0.72 ± 0.15 nA/μM. In addition, the detection limit for the designed glucose biosensor system has been observed to be about 0.35 μM. The limit was calculated following the procedure reported by *Susana et al.* [40] using the values of the slope of the linear calibration plot and the standard deviation. The standard deviation was estimated from the amperometric signals (n = 10) of 8 mM glucose.

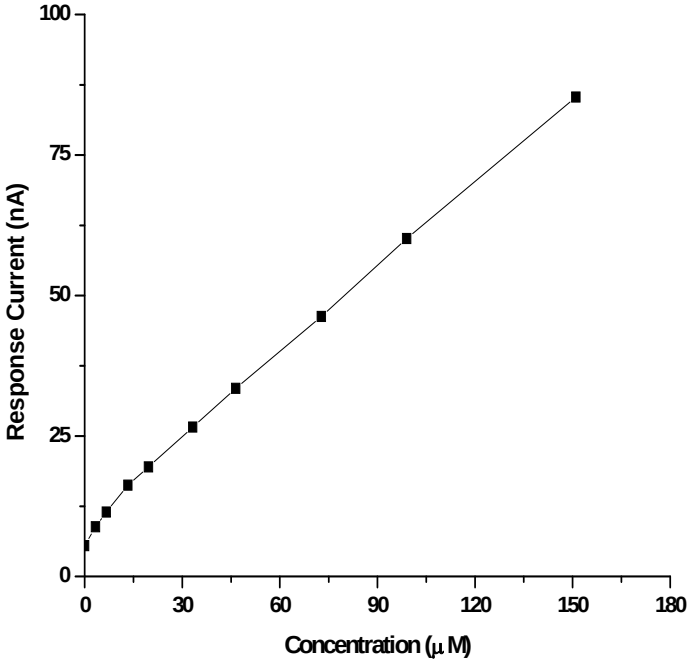


Fig. 4. Calibration curve of the enzyme electrode for glucose.

3.3. pH Dependence

The pH dependence of the buffer medium on the amperometric response to the glucose of the enzyme electrode was studied over the clinically and commercially relevant range. The pH optimum of an immobilized enzyme may not be the same as that of the soluble enzyme. Bound to a negatively charged matrix, the pH optimum shifts to higher value, while on a positively charged support, the converse may be true [41].

Fig. 5 shows the biosensor response against the pH of the buffer solution used. The highest response was observed at pH 6.8 which was subsequently utilized in further studies. However, it is important to

note that the glucose assay is based up on the electrochemical oxidation of the hydrogen peroxide which itself is a pH dependent redox process. The pH dependence of the biosensor system might also be due to the contribution of the polymer matrix's amphiphilic characteristics in the micro environment of the enzyme near the bulk electrode.

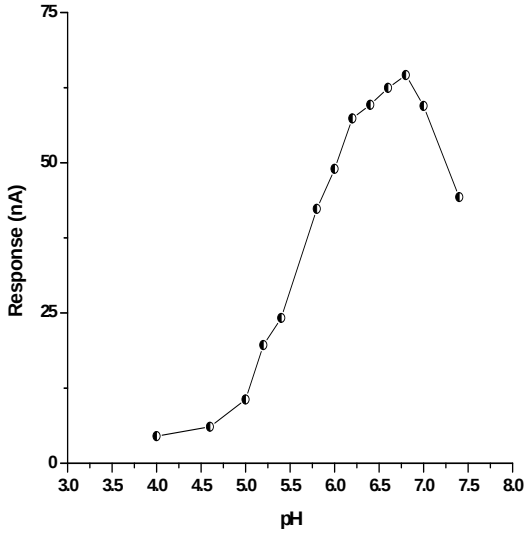


Fig. 5. Influence of pH on the response of the glucose biosensor.

3.4. Temperature Dependence

The effect of temperature of the buffer solution on the response of the Pt/PCS+PEI/GOD glucose biosensor system was studied in the range of 283 – 333 K (Fig. 6). It is seen that the amperometric response initially increases with the temperature and decreases later. The response reached a maximum at about 308 K. The decrease of the response after 308 K may be attributed to the thermal inactivation of the enzyme or the enhanced disproportionate kinetics of hydrogen peroxide at higher temperatures which is favored over the electrochemical oxidation at the platinum electrode [42].

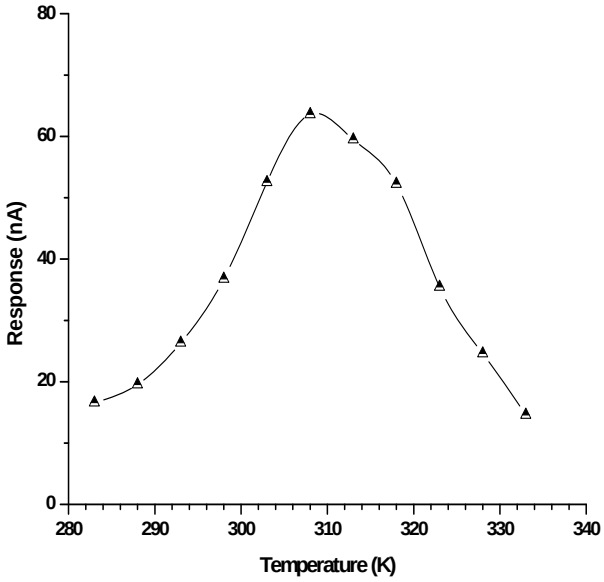


Fig. 6. Effect of temperature on the response of the glucose biosensor.

3.5. Specificity, Selectivity and Interference

In order to confirm the specificity of the enzyme electrode, the amperometric response to glucose injections without the enzyme were checked. Since, no measurable amperometric response could be observed, it has been concluded that the enzyme layer in polymer matrix was not only essential but also responsible for the observed results. The use of enzyme based amperometric glucose biosensors for determining substrates in complex media, like biological fluids, poses some problems such as the interference of electro active species giving a Faradic response at the working potential or fouling of the electrode surface [20]. The interference of electro active species can be decreased by using a mediator to decrease the working potential. In the present investigation, a working potential of +600 mV against Ag/AgCl was used for the glucose detection. At such a potential, there may be interferences from other oxdisable species such as ascorbic acid (AA), uric acid (UA), acetaminophen (AP) etc.

One approach to avoid interferents and improve the selectivity is the use of selective membranes that are permeable for hydrogen peroxide but not permeable to interferents' spices like AA, UA, AP etc. in glucose detection. In general, the selectivity of the polymer/membrane used in the enzyme immobilization depends on its pore size and charges [22]. Recently, electropolymerized non-conducting polymer films have been used to develop enzyme based amperometric biosensors [43]. The non-conducting polymers normally exhibit good selectivity and fast response and, therefore, have attracted much attention recently. However, their use is still a matter of concern in the biosensor research because of their relatively high detection limit and low response currents. To increase the response current, ferrocene, an electron transfer mediator for the amperometric hydrogen peroxide sensor, has been used in the biosensors based on the non-conducting polymer [39, 44-45]. Hence, in order to mitigate the interference effects, the recent glucose biosensor systems utilize electro polymerization of non-conducting polymers with artificial mediators, making the whole process still more complex.

The authors, in the present investigation, utilized a unique but a simple to synthesize polymer matrix of PCS+PEI without any artificial mediator. The system was then subjected to interference tests by adding AA and UA in to the biosensor cell system. The sensor was found to respond successfully to glucose injections in the presence of these interferents. The interfering effects were estimated as a percentage of difference in the biosensor signal recorded in the presence of the interferents. The difference in the response current was found within 3 % of relative standard deviation (RSD). The PCS+PEI polymer matrix used in the present investigation has been found to act as an effective barrier to protect the electrodes from the AA and UA interference as well as from the fouling during the glucose detection. Probably the size exclusion and/or anion–exclusion capabilities of the unique PCS+PEI matrix resulted in the suppression of the interferences. The antifouling property of the polymer matrix should make the present sensor system highly suitable for the determination of glucose very effectively in biological fluids such as blood or serum and also in fruit juices and other food products.

3.6. Storage Stability and Shelf Life

Most enzymes lose their activity when not stored in refrigerator, and therefore, storage at low temperature (+4 ^{o}C) is one of the most important parameters to retain the stability of glucose biosensors. Moreover, in order to attain high water content in the immobilized layer, the sensors were kept in a closed bottle together with wet cotton to achieve moisture condition. Fig. 7 compares the storage stability of the two enzyme sensors under different moist conditions of the refrigerator.

The operational stability of a biosensor response may vary considerably depending upon the sensor geometry, method of preparation, biological recognition reactions etc. Although some biosensors have been reported to be usable under laboratory conditions for more than one year, their *practical life time* is either unknown or limited to a few days/weeks when they are incorporated in to practical use.

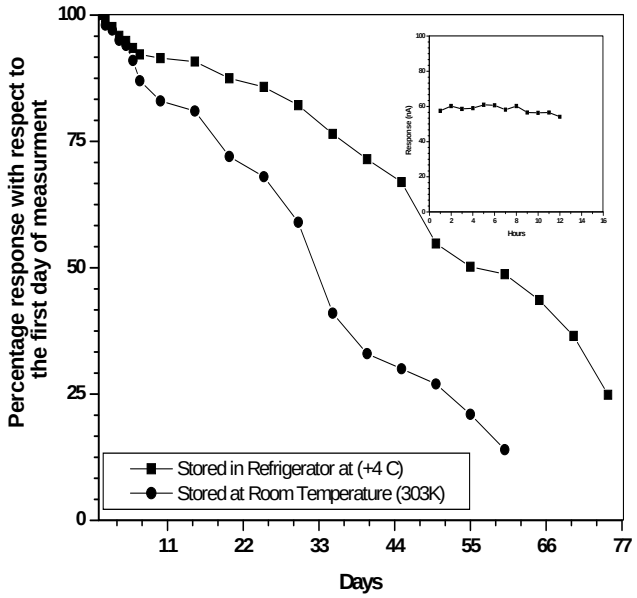


Fig. 7. Storage stability of Pt/PCS+PEI/GOD glucose biosensor. Inset shows continuous operation of the biosensor.

The life time of the biosensor of the present investigation was found to be in the range of 5-6 days and a slight decrease in electrode response was observed later, in a continuous operation. This life time is quite suitable for disposable types of biosensors. A gradual decrease in the amperometric current was observed after an average of 25 to 30 measurements (at room temperature of 303 K) of 12 Hours/Day continuous operation. An untested Pt/PCS+PEI/GOD biosensor system was found to remain intact for about four months or so. A prudent use, maximum 12 hours/day operation and storage at very low temperatures, enabled the sensor system to function properly up to about 60-75 days.

3.7. Real Samples

The fabricated enzyme based glucose biosensor has been then tested for real sample analysis by using a commercial soft drink of ‘fanta’, ‘cola’, fruit juices of orange and apple and also human blood samples collected from hospitals. The results obtained for the fruit juices and soft drinks were compared with those obtained with HPLC test. The content of glucose in the blood samples determined by the biosensor was compared with those measured by the biochemical analyzer of the hospital. Table 1 summarizes the results obtained during the present investigation.

The results indicate that the performance of the Pt/PCS+PEI/GOD biosensor system is at par with the commercially accepted methods.

In the present case, the GOD was immobilized in a polymer matrix of PEI and PCS hydrogel. The combination of PCS hydrogel and the layer forming PEI were found to offer a better enzyme entrapment instead of the layer only type of entrapment by the reported electropolymerized polymers. The authors believe that the present gel entrapment based GOD biosensor offers a simpler option

compared to that of cross linking based biosensor system. Cross linking an enzyme to itself is both expensive and inefficient as some of the protein material will inevitably be acting as a support and resulting in a relatively low enzyme activity. In addition, the cross linking immobilization procedure has a disadvantage of harsh treatment of bio catalyst by toxic chemicals [22]. Hence, the PCS gel entrapment based glucose biosensor may be viewed as a low cost, easy to fabricate, simple to operate, portable and disposable type.

Table 1. Comparison of Glucose determined by using the fabricated biosensor and commercial HPLC/ Hospital test method.

Samples		**Glucose Concentration determined by Pt/PCS+PEI/GOD Biosensor**	**Glucose Concentration determined by HPLC (fruit juice)/ Hospital Test (human blood)**
Fruit Juice / Soft Drink			
1.	Orange Juice	23.41 ± 0.24 mg/dL	24.08 mg/dL
2.	Apple Juice	24.36 ± 0.20 mg/dL	24.93 mg/dL
3.	Fanta Soft drink	27.59 ± 0.12 mg/dL	27.32 mg/dL
4.	Cola Soft drink	21.78 ± 0.12 mg/dL	22.32 mg/dL
Human Blood			
5.	Sample: 1	4.48 mM	4.36 mM
6.	Sample: 2	4.12 mM	3.93 mM
7.	Sample: 3	6.19 mM	6.52 mM
8.	Sample: 4	5.48 mM	5.73 mM
9.	Sample: 5	8.76 mM	8.44 mM

4. Conclusions

A low cost, easy to fabricate, simple to operate and a disposable type of glucose biosensor based upon GOD enzyme immobilized in a unique polymer matrix of PCS+PEI has been developed and reported. The sensitivity of the designed and fabricated glucose biosensor has been found to be 0.72 ±0.15 nA/μM. In addition, the lower detection limit for the glucose biosensor has been observed to be about 0.35 μM of glucose. The PCS+PEI polymer matrix used in the present investigation has been found to be an effective barrier to the interferents such as ascorbic acid (AA) and uric acid (UA). The Pt/PCS+PEI/GOD glucose biosensor system has been tested for the determination of glucose concentration in real samples of fruit juice, soft drinks and human blood. The results indicated a close agreement with those obtained by HPLC and hospital test methods. The developed Pt/PCS+PEI/GOD glucose biosensor system without the presences of an artificial mediator could offer a rapid and reproducible method for the specific determination of glucose in the real samples.

References

[1]. Tran Min Canh, Biosensors, *Chapman & Hall and Masson*, London, UK, 1993.
[2]. R. C. Bateman, J. A. Evans, Using the glucose oxidase/peroxidase system in enzyme kinetics, *J. Chem. Educ.*, Vol. 72, 1995, pp. 240-241.
[3]. F. Cespedes, F. Valero, E. Martinez-Fabregas, J. Bartroli, S. Alegret, Fermentation monitoring using a glucose biosensor based on an electrocatalytically bulk-modified epoxy–graphite biocomposite integrated in a flow system, *Analyst*, Vol. 120, 1995, pp. 2255.
[4]. Jianguo Liu, Gaoxiang Li, Shunzhen Xie, Rushui Han, Kemin Chen, Biosensor for fermentation process control, *Enzyme Engineering XIV*, Vol. 864, 1998, pp. 59-69.

[5]. T. C. Mullis, J. T. Winge, S. T. Deal, An alternative producer for the glucose oxidase assay of glucose as a applied to the lactase activity assay, *J. Chem. Educ.*, Vol. 76, 1999, pp. 1711-1718.
[6]. P. L. Edmiston, T. R. Williams, An analytical experiment in error analysis; Repeated determination of glucose using commercial glucometers, *J. Chem. Educ.*, Vol. 77, 2000, pp. 377-379.
[7]. Wang H, Wang X, Zhang X, Qin X, Zhao Z, Miao Z, Huang N, Chen Q., A novel glucose biosensor based on the immobilization of glucose oxidase onto gold nanoparticles-modified Pb nanowires, *Biosens. Bioelectron.*, Vol. 15, 2009, pp. 142.
[8]. HaiLing Chen, Li Zhao, Xi Chen, ZhiXia Zhuang and XiaoRu Wang, Development of an amperometric glucose biosensor based on the immobilization of glucose oxidase in an ormosil-PVA matrix onto a Prussian Blue modified electrode, *Science in China Series B: Chemistry*, Vol. 52, 2009, pp. 1128.
[9]. A. Han, G. Wu, Q. Liang, F. Zhang, L. Hao, X. Zhang, Y. Tang, Direct Spectrophotometric Determination of the Blood Glucose by the Method of Conjugation Enzyme, *Fenxi Huaxue*, Vol. 31, 2003, pp. 1417.
[10].F. Luo, Y. Li, H. Han, Determination of glucose in Compound Dansheng injection by polarimetry, *West China J. Pharm. Sci.*, Vol. 18, 2003, pp. 147.
[11].H. Nakamura, I. Karube, Current research activity in biosensors, *Anal. Bioanal. Chem.*, Vol. 377, 2003, pp. 446-468.
[12].J-M. Zen, Y-S. Song, H-H. Chung, C-T. Hus, A-S. Kumar, Photoelectrochemical oxygen sensor using copper plated screen printed carbon electrodes, *Anal. Chem.*, Vol. 74, 2002, pp. 6126-6131.
[13].J. D. Newman, A. P. F. Turner, Home blood glucose biosensors: a commercial perspective, *Biosensor. Bioelectron.*, Vol. 20, 2005, pp. 2435-2453.
[14].H. Wei, J. J. Sun, Y. Xie, C. G. Lin, Y. M. Wang, W. H. Yin, G. N. Chen., Enhanced electrochemical performance at screen printed carbon electrodes by a new pre-treating procedure, *Anal. Chim. Acta.*, Vol. 588, 2007, pp. 297-303.
[15].C. H. Lee, S. C. Wang, C. J. Yuan, M. F. Wen, K. S. Chang, Comparison of amperometric biosensors fabricated by palladium sputtering, palladium electrodepostion and nafion/carbon nanotube casting on screen printed carbon electrodes, *Biosens. Bioelectron.*, Vol. 22, 2007, pp. 877-884.
[16].D. Shan, S. Wang, Y. He, H. Xue, Amperometric glucose biosensor based on in situ electropolymerized polyaniline/poly(acrylonitrile-co-acrylic acid) composite film, *Materials Science and Engineering*, Vol. C28, 2008, pp. 213-217.
[17].T. H. Cheng, H. H. Chung, D. M. Taai, M. Y. Fang, H. C. Hsaio, J. M. Zen, Fabrication of glucose biosensor based on inserted barrel plating gold electrodes, *Anal. Chem.*, Vol. 81, 2009, pp. 515-518.
[18].K. Abu-Rabeah, R. S. Marks, Impedance study of the hybrid molecule alginate-pyrrole: Demonstration as host matrix for the construction of a highly sensitive amperometric glucose biosensor, *Sensors and Actuators B*, Vol. 136, 2009, pp. 516-522.
[19].H. J. Wang, C. M. Zhou, F. Peng, H. Yu., Glucose biosensor based on platinum nanoparticles supported sulfonated-carbon nanotubes modified glassy carbon electrode, *Int. J. Electrochem. Sci.*, Vol. 2, 2007, pp. 508-516.
[20].W. Z. Jia, K. Wang, X. H. Xia, Elimination of electrochemical interferences in glucose biosensors, *Trends in Analytical Chemistry*, Article in Press and available online, 2010.
[21].M. Ozden, E. Ekinci, A. E. Karagozier, Electrochemical preparation and sensor properties of conducting polyaniline films, *Turk. J. Chem.*, Vol. 23, 1999, pp. 89-98.
[22].M. Gerard, A. Chaubey, B. D. Malhotra, Review: Application of conducting polymers to biosensors, *Biosensor and Bioelectronics*, Vol. 17, 2002, pp. 345-359.
[23].M. Mehrvar, M. Abdi, Recent developments, characteristics, and potential applications of electrochemical biosensors, *Analytical Science*, Vol. 20, 2004, pp. 1113-1126.
[24].H. H. Zhou, H. Chen, J. H. Chen, Y. F. Kuang, Polyaniline-graphite composite film glucose oxidase electrode, *J. Cent. South Unvi. Technol.*, Vol. 13, Issue 6, 2006, pp. 653.
[25].A. Ramanavicius, A. Kausaite, A. Ramanaviciene, Polypyrrole-coated glucose oxidase nanoparticles for biosensor design, *Sensor and Actuator B*, Vol. 111-112, 2005, pp. 532-539.
[26].J. C. Vidal, E. Garcia, S. Mendez, P. Yarnoz, J. R. Castillo, Three approach to the development of selective bi-layer amperometric biosensors for glucose by in situ electropolymerization, *Analyst*, Vol. 124, 1999, pp. 319-324.
[27].M. Yuqing, C. Jianrong, W. Xiaohua, Construction of a glucose biosensor by immobilizing glucose oxidase within a poly(o-phenylenediamine) covered screen printed electrode, *J. Biological Sci.*, Vol. 6, 2006, pp. 18-22.
[28].X. G. Li, M. R. Huang, Y. Yang, Synthesis and characterization of o-phenylenediamine and xylidine copolymers, *Polymer*, Vol. 42, 2001, pp. 4099-4107.

[29].M. S. Topcu, O. Gokdogan, A. Gulce, H. Gulce, Amperometric glucose biosensor based on gold-deposited polyvinylferrocene film on Pt electrode, *Biosens. Bioelectron*, Vol. 21, 2006, pp. 1719.
[30].W. W. Yang, J. X. Wang, S. Zhao, Y. Y. Sun, C. Q. Sun, Multilayered construction of glucose oxidase and gold nanoparticles on Au electrodes based on layer-by-layer covalent attachment, *Electrochem. Commun.*, Vol. 8, 2006, pp. 665.
[31].C. P. Remirez, D. J. Chruana, Immobilisation of glucose oxidase in electrodeposited copper, *Electrochem. Commun.*, Vo. 8, 2006, pp. 450.
[32].Y. C. Tsai, S. C. Li, J. M. Chen, Cast thin film biosensor design based on a nafion backbone, a multi-walled carbon nanotube conduit, and a glucose oxidase function, *Langmuir*, Vol. 21, 2005, pp. 3653-3659.
[33].Y. Lui, M. K. Wang, F. Zhao, Z. A. Xu, S. J. Dong, The direct electron transfer of glucose oxidase and glucose biosensor based on carbon nanotubes/chitosan matrix, *Biosens. Bioelectron*, Vol. 21, 2005, pp. 984.
[34].H. J. Wang, C. M. Zhou, F. Peng, H. Yu, Glucose biosensor based on platinum nanoparticals supported sulfonated carbon nanotubes modified glassy carbon electrode, *Int. J. Electrochem Sci.*, Vol. 7, 2007, pp. 508-516.
[35].H. Wu, J. Wang, X. Kang, C. Wang, D. Wang, J. Liu, I. A. Aksay, Y. Lin, Glucose biosensor based on immobilization of glucose oxidase in platinum nanoparticles/ graphene/chitosan nanocompostie film, *Talanta*, Vol. 80, 2009, pp. 403-406.
[36].L. C. Clark, C. Lyons, Electrode systems for continuous monitoring in cardiovascular surgery, *Acad. Sci.*, Vol. 102, 1962, pp. 29-35.
[37].U. B. Trivedi, D. Lakshminarayana, I. L. Kothari, P. B. Patel, C. J. Panchal, Amperometric fructose biosensor based on fructose dehydrogenase enzyme, *Sensors and Actuators B*, Vol. 136, 2009, pp. 45-51.
[38].U. B. Trivedi, D. Lakshminarayana, I. L. Kothari, N. G. Patel, H. N. Kapse, K. K. Makhija, P. B. Patel, C. J. Panchal, Potentiometric biosensor for urea detection in milk, *Sensors and Actuators B*, Vol. 140, 2009, pp. 260-266.
[39].G. Fortier, E. Brassard, D. Belanger, Fast and easy preparation of an amperometric glucose biosensor, *Biotechnol. Tech.*, Vol. 2, 1988, pp. 177-182.
[40].Susana Campuzana, Rocio Galvez, Maria Pedrero, F. Javier Manuel de Villena, Jose M. Pingarrom, An integrated electrochemical fructose biosensor based on tetrathiafulvalene-modified self assembled monolayers on gold electrodes, *Anal. Bioanal. Chem.*, Vol. 377, 2003, pp. 600.
[41].W. S. Kisaalita, Biosensors standards requirements, *Biosensors and Bioelectronics*, Vol. 7, 1992, pp. 613-620.
[42].G. Fortier, E. Brassard, D. Belanger, Optimization of a polypyrrole glucose biosensor, *Biosensors and Bioelectronics*, Vol. 5, 1990, pp. 473-490.
[43].Miao Yuqing, Chen Jianrong, Wu Xiaohua, Using electropolymerized non conducting polymers to develop enzyme amperometric biosensors, *Trends in Biotechnology*, Vol. 22, 2004, pp. 227-231.
[44].J. Wang, L. Chen, S. B. Hocevar, B. Ogoreve, One step electropolymeric co immobilization of glucose oxidase and heparin for amperometric biosensing of glucose, *The Analyst*, Vol. 125, 2000, pp. 1431-1434.
[45].C. X. Li, Y. L. Zeng, C. R. Tang, Glucose biosensor based on carbon/PVC-COOH/Ferrocene composite with covalently immobilized enzyme, *Chinese Chemical Letters*, Vol. 16, 2005, pp. 1357-1360.

Guide for Contributors

Aims and Scope

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.

Topics Covered

Contributions are invited on all aspects of research, development and application of the science and technology of sensors, transducers and sensor instrumentations. Topics include, but are not restricted to:

- Physical, chemical and biosensors;
- Digital, frequency, period, duty-cycle, time interval, PWM, pulse number output sensors and transducers;
- Theory, principles, effects, design, standardization and modeling;
- Smart sensors and systems;
- Sensor instrumentation;
- Virtual instruments;
- Sensors interfaces, buses and networks;
- Signal processing;
- Frequency (period, duty-cycle)-to-digital converters, ADC;
- Technologies and materials;
- Nanosensors;
- Microsystems;
- Applications.

Submission of papers

Articles should be written in English. Authors are invited to submit by e-mail editor@sensorsportal.com 8-14 pages article (including abstract, illustrations (color or grayscale), photos and references) in both: MS Word (doc) and Acrobat (pdf) formats. Detailed preparation instructions, paper example and template of manuscript are available from the journal's webpage: http://www.sensorsportal.com/HTML/DIGEST/Submition.htm Authors must follow the instructions strictly when submitting their manuscripts.

Advertising Information

Advertising orders and enquires may be sent to sales@sensorsportal.com Please download also our media kit: http://www.sensorsportal.com/DOWNLOADS/Media_Kit_2009.pdf