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# Preparation of PVA membrane for immobilization of GOD for glucose biosensor

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**Abstract**

A membrane was prepared using polyvinyl alcohol (PVA) with low and high degree of polymerization (DOP), acetone, benzoic acid (BA) and was cross-linked by UV treatment. Membrane composition was optimized on the basis of swelling index. Membrane prepared with 12% low DOP and 8% high DOP of PVA, 2% BA, dissolved in buffer containing 20% acetone and cross-linked with UV treatment exhibited lower swelling index. Fourier transform infrared (FTIR) study of the membranes showed appearance of a strong band at ~2337 $cm^{-1}$ when UV was used for cross-linking in the presence of benzoic acid. Scanning electron microscope (SEM) study revealed that membrane cross-linked with UV treatment was smoother. Glucose oxidase (GOD)–PVA membrane was associated with the dissolved oxygen (DO) probe for biosensor reading. Glucose was detected on the basis of depletion of oxygen, when immobilized GOD oxidizes glucose to gluconolactone. A wide detection range, 0.9–225 mg/dl was estimated from the linear range of calibration plot of biosensor reading. Membranes were reused for 32 reactions without significant loss of activity and stored for 30 days (~90% activity) at 4 °C. Membranes were also used with real blood samples.


*Keywords:* PVA membrane; Swelling index; FTIR study; Glucose oxidase; Dissolved oxygen; Glucose biosensor

## 1. Introduction

The basic requirement of a biosensor is that the biological material should bring the physico-chemical changes in close proximity of a transducer. In this direction, immobilization technology has played a major role [1–3]. Immobilization not only helps in forming the required close proximity between the biomaterial and the transducer, but also helps in stabilizing it for reuse. The biological material has been immobilized directly on the transducer or in most cases, in membranes, which can subsequently be mounted on the transducer. Biomaterials can be immobilized either through adsorption, entrapment, covalent binding, cross-linking or a combination of all these techniques [3–5]. A number of techniques have been developed in our laboratory for the immobilization of enzymes as well as cells for this purpose [3,6,7]. Generally, entrapment is considered as a suitable method for enzyme immobilization because of mild coupling procedure.

Synthetic polymer that has been extensively used for immobilization of biocatalysts in a membranous form is polyvinyl alcohol (PVA). It is a non-toxic and biocompatible synthetic polymer with good chemical and thermal stability [8]. Large numbers of hydroxyl groups in the PVA provide a biocompatible microenvironment for the enzyme [9]. However major limitation of PVA membrane is its high swelling index and dissolves readily in water when not cross-linked [10]. Swelling of the PVA membrane was reduced by cross-linking with a variety of chemical and physical methods [11,12]. In our laboratory, urease enzyme was entrapped in the composite polymer membrane of polyvinyl alcohol–polyacrylamide and cross-linked by γ-irradiation and was reused for a number of times without any significant loss of urease activity [13].

We report the preparation of a membrane containing glucose oxidase (GOD), using PVA with low and high degree of polymerization (DOP), acetone as a mixture of solvent, benzoic acid (BA) as sensitizer and cross-linked using UV treatment.

Glucose oxidase was selected for immobilization since it is a well studied and applicable in the field of biosensor [14,15]. Most of glucose biosensors are based on oxidation of glucose

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catalysed by glucose oxidase:

$$\text{D-Glucose} + O_2 \xrightarrow{\text{GOD}} H_2O_2 + \text{D-Gluconolactone}$$

The amperometric response of consumed $O_2$ was monitored for glucose oxidation using oxygen-sensitive electrode [16].

Composition for preparation of membrane was optimized on the basis of the swelling index. Fourier transform infrared (FTIR) and scanning electron microscope (SEM) studies of the membranes were carried. Operational reusability as well as stability of the GOD–PVA membrane was studied. The applicability of the membrane was confirmed with real blood samples.

## 2. Materials and methods

### 2.1. Materials

Polyvinyl alcohol (degree of hydrolysis 98–99%) with DOP ~360 and 1700–1800 were purchased from Fluka Chemie, GmbH. Benzoic acid from Merck, Mumbai, India. Glucose oxidase (E.C.1.1.3.4), type II from *Aspergillus niger* lyophilized powder (100 units/mg protein) from Sisco Research Laboratory, Mumbai. Glucose, acetone and other chemicals were obtained from standard sources. Clark dissolved oxygen (DO) electrode (probe) was purchased from M/S Century Instruments, Chandigarh, India. Blood samples and their glucose values were provided by the pathology department of BARC Hospital, Anushaktinagar, Mumbai.

### 2.2. Optimization of PVA membrane composition

Different compositions of PVA with low (~360) and high (1700–1800) DOP and benzoic acid were dissolved in milliQ water containing different percentages of acetone (Table 1). The mixtures were dissolved homogenously at 70–80 °C on heating magnetic stirrer with vigorous stirring. PVA mixture was allowed to cool to room temperature and overlaid as a thin membranous layer on the lid of a Petri plate in order to obtain required thin and uniform membranes. Membranes were air-dried for 2 h. Dried membranes were peeled off carefully and were cross-linked by exposure to UV light for 1 h.

Table 1
Compositions of the membrane and their swelling index

| Membrane compositions | Swelling index (%) |
|---|---|
| 12% LDOP PVA + 8% HDOP PVA (no UV treatment) | 103 ± 10.8 |
| 12% LDOP PVA + 8% HDOP PVA + UV treatment | 96.7 ± 10.4 |
| 12% LDOP PVA + 8% HDOP PVA + 20% acetone as solvent (no UV treatment) | 89.65 ± 9.5 |
| 12% LDOP PVA + 8% HDOP PVA + 20% acetone as solvent + UV treatment | 87.8 ± 9.2 |
| 12% LDOP PVA + 8% HDOP PVA + 2% BA (no UV treatment) | 70.17 ± 7.1 |
| 12% LDOP PVA + 8% HDOP PVA + 2% BA + UV treatment | 33.6 ± 3.5 |
| 12% LDOP PVA + 8% HDOP PVA + 2% BA + 20% acetone as solvent + UV treatment | 26.3 ± 3.1 |

LDOP PVA: Polyvinyl alcohol of low degree of polymerization (~360). HDOP PVA: Polyvinyl alcohol of high degree of polymerization (~1700–1800). BA: Benzoic acid. UV treatment: Ultra violet light (irradiation) treatment for 1 h.

### 2.3. Swelling index of the PVA membrane

Circular pieces of the membranes were cut and weighed. The pieces were then immersed in milliQ water for 24 h at room temperature to attain the equilibrium sorption. Surface adhered water drops were wiped using soft filter papers after attainment of equilibrium and weighed immediately. Weights were noted down, after and before water treatment. Swelling index ($S$) was calculated according to equation [10,17]:

$$S(\%) = \frac{W_s - W_d}{W_d} \times 100$$

where $W_s$ and $W_d$ are the weight of wet membrane at equilibrium of sorption and the weight of dry membrane respectively. Inferences were made for the quality of the membranes on the basis of swelling index.

### 2.4. FTIR study of the membrane

Membranes were studied by FTIR spectra and scanned in the range of 4000–400 $cm^{-1}$ on Jasco (Model FTIR-660 plus) FTIR Spectrometer. The membranes were pressed directly onto the attenuated reflectance crystal KBr (KBr was supplied with FTIR instrument) with the sampling unit.

### 2.5. SEM study of the membrane

A scanning electron microscope (Model 435 VP, Leo Electron Microscopy Ltd., Cambridge, UK) was employed to observe the surface of the PVA membranes. For SEM, pieces of the membranes were mounted on stubs and were then coated with gold using a sputter coater. The SEM micrographs of the PVA membranes (before and after the UV treatment) were taken at magnifications 100, 500 and 2000×.

### 2.6. GOD immobilization in PVA membrane

A 10 ml homogenous mixture of 12% LDOP PVA, 8% HDOP PVA and 2% BA was prepared in 50 mM sodium phosphate buffer (pH 6.0) containing 20% acetone and 2 ml GOD (2000 units/ml) was added to the mixture and mixed homogenously. The mixture was overlaid as a thin membranous layer, air-dried, peeled off carefully and placed under UV light for cross-linking. Cross-linked GOD–PVA membranes were stored at 4 °C.

### 2.7. Operating condition and calibration of the DO probe

Commercial Clark-type dissolved oxygen electrode in conjunction with GOD entrapped PVA membrane was used for

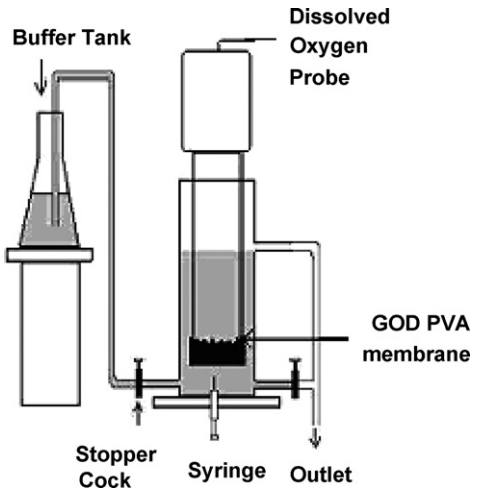


Fig. 1. Schematic diagram of association of GOD–PVA membrane with the DO probe biosensor.

biosensor reading as shown in Fig. 1. The electrode was filled with 1 M potassium chloride solution. Blank readings of the probe with air and 50 mM sodium phosphate buffer (pH 6.0) were taken without the membrane being attached to the electrode. Membrane was tightly attached to the electrode using O-ring with the support of cheesecloth. The DO probe was allowed to obtain a base line corresponding to the buffer volume 20 ml in the reaction vessel. 500 μl of standard glucose solutions (concentration 0.9, 4.5, 22.5, 90, 225 and 450 mg/dl) were introduced into the reaction vessel and oxidation reaction occurred at electrode surface was observed. Oxygen was consumed from sample during oxidation of glucose and depletion of oxygen was measured using DO probe. All experiments were performed at room temperature.

## 3. Results and discussions

### 3.1. Optimization of composition for preparation of PVA membrane

Membranes were prepared using low and/or high degrees of polymerized PVA, acetone, and benzoic acid and were cross-linked by UV treatment. Membrane composition was optimized on the basis of swelling index. Swelling index was reduced after cross-linking membrane with UV treatment in presence of benzoic acid as sensitizer (Table 1) and the result is better than the earlier report [9]. This shows that the stability of the membrane was improved because of cross-linking.

Swelling properties was further reduced when the membrane was prepared using both low and high degree of polymerized PVA. From the study, it was observed that membrane prepared with solution comprising 12% LDOP PVA and 8% HDOP PVA was having lower swelling index (32.5 ± 4.1%) than the either only 20% low (53.4 ± 5.6%) or only 20% high (55.3 ± 5.5) DOP of PVA. Membrane properties, flexibility and tensile strength were improved when mixture of both, low and high DOP of PVA was used for preparation of membrane. Flexibility of the membrane increased with decrease of degree of polymerization

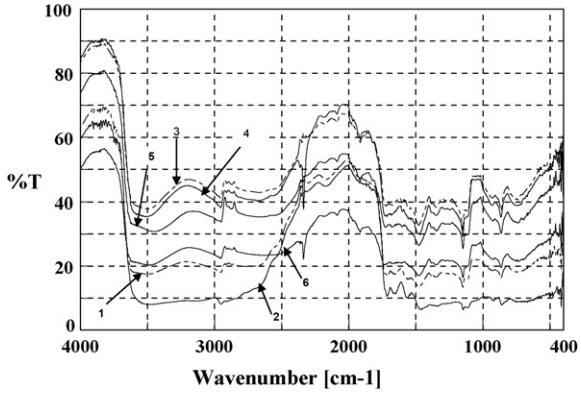


Fig. 2. FTIR spectrum studies of the membranes prepared using mixtures of the compositions given below: (1) 12% LDOP PVA + 8% HDOP PVA (no UV treatment). (2) 12% LDOP PVA + 8% HDOP PVA + UV treatment. (3) 12% LDOP PVA + 8% HDOP PVA + 2% BA (no UV treatment). (4) 12% LDOP PVA + 8% HDOP PVA + 2% BA + UV treatment. (5) 12% LDOP PVA + 8% HDOP PVA + 2% BA + 20% acetone as solvent (no UV treatment). (6) 12% LDOP PVA + 8% HDOP PVA + 2% BA + 20% acetone as solvent + UV treatment.

of the PVA and tensile strength of the membrane increased with increase of DOP of the PVA [18].

Swelling index was further reduced by using solvent with 20% acetone for preparation of membrane as shown in Table 1.

Above study indicated that the membrane comprised of 12% LDOP and 8% HDOP of PVA along with sensitizer (2% BA), dissolved in buffer containing 20% acetone and cross-linked with the UV treatment was having lowest swelling property (Table 1). This composition was used further for preparation of membrane and immobilization of enzyme for biosensor development.

### 3.2. FTIR study of the membranes

FTIR spectra of PVA membranes with different composition are shown in Fig. 2 and their peaks were assigned in Table 2 [19–21]. It was observed that a strong band appeared at ∼2337 $cm^{-1}$ after UV cross-linking in the presence of benzoic acid and same band was absent when either benzoic acid or UV treatment was absent. The band appeared at ∼2337 $cm^{-1}$ was corresponding to the free $CO_2$ gases entrapped inside the alkoxide polymer [20]. It was earlier reported that the UV treatment could be used for cross-linking in presence of benzoate [9], where benzoate acted as a sensitizer. The cross-linking was always accompanied by photolysis of the sensitizer and in the absence of sensitizer, no cross-linking occurred. Probable mechanism was reported in literature [22], a free radical arising from the photolysis of benzoate would abstract a tertiary hydrogen atom from the polymer chain to yield a polymeric radical, which can cross-link by combination with another such radical.

### 3.3. SEM study of the membranes

SEM study of the surface of PVA membranes before and after UV treatment was carried out. It was observed that surface of the membranes after UV treatment was having less ridges

Table 2
FTIR peaks assignment for PVA membranes

| Wave number ($cm^{-1}$) | | | | | | Peak assignments |
|---|---|---|---|---|---|---|
| 1 | 2 | 3 | 4 | 5 | 6 | |
| ∼2954 | ∼2951 | ∼2954 | ∼2954 | ∼2951 | ∼2953 | Asymmetrical stretching vibration of methylene ($\nu_{as}CH_2$ and CH aliphatic) |
| | | | | ∼2876 | | C–H stretching of the symmetric methyl band, $\nu s(CH_3)$ |
| | | | ∼2337 | | ∼2337 | C═O stretching of $CO_2$. Free $CO_2$ gas was permanently entrapped within polymer during cross-linking |
| | | ∼1914 | ∼1914 | ∼1914 | ∼1914 | Vibration stretching of C═O bands due to excited state |
| ∼1715 | ∼1715 | ∼1723 | ∼1716 | ∼1729 | ∼1729 | Vibration stretching of C═O |
| ∼1472 | ∼1456 | ∼1479 | ∼1473 | ∼1473 | ∼1472 | Methylene deformation $\delta(CH_2)$ |
| ∼1341 | ∼1345 | ∼1345 | ∼1348 | ∼1346 | ∼1348 | Stretching C═O and methylene deformation |
| ∼1149 | ∼1148 | ∼1150 | ∼1149 | ∼1148 | ∼1149 | Before $CO_2$ adsorption, a band assigned to the C–H bending mode. C–O–C asymmetric and symmetric stretching and C═O stretching vibration and C–H bending and rocking mode |
| ∼865 | ∼860 | ∼864 | ∼865 | ∼864 | ∼864 | Deformation of a anomeric C–H vibration |
| ∼748 | ∼669 | ∼739 | ∼741 | ∼740 | ∼738 | Skeletal mode of vibration of anomeric carbons |

(1) 12% LDOP PVA + 8% HDOP PVA (no UV treatment). (2) 12% LDOP PVA + 8% HDOP PVA + UV treatment. (3) 12% LDOP PVA + 8% HDOP PVA + 2% BA (no UV treatment). (4) 12% LDOP PVA + 8% HDOP PVA + 2% BA + UV treatment. (5) 12% LDOP PVA + 8% HDOP PVA + 2% BA + 20% acetone as solvent (no UV treatment). (6) 12% LDOP PVA + 8% HDOP PVA + 2% BA + 20% acetone as solvent + UV treatment.

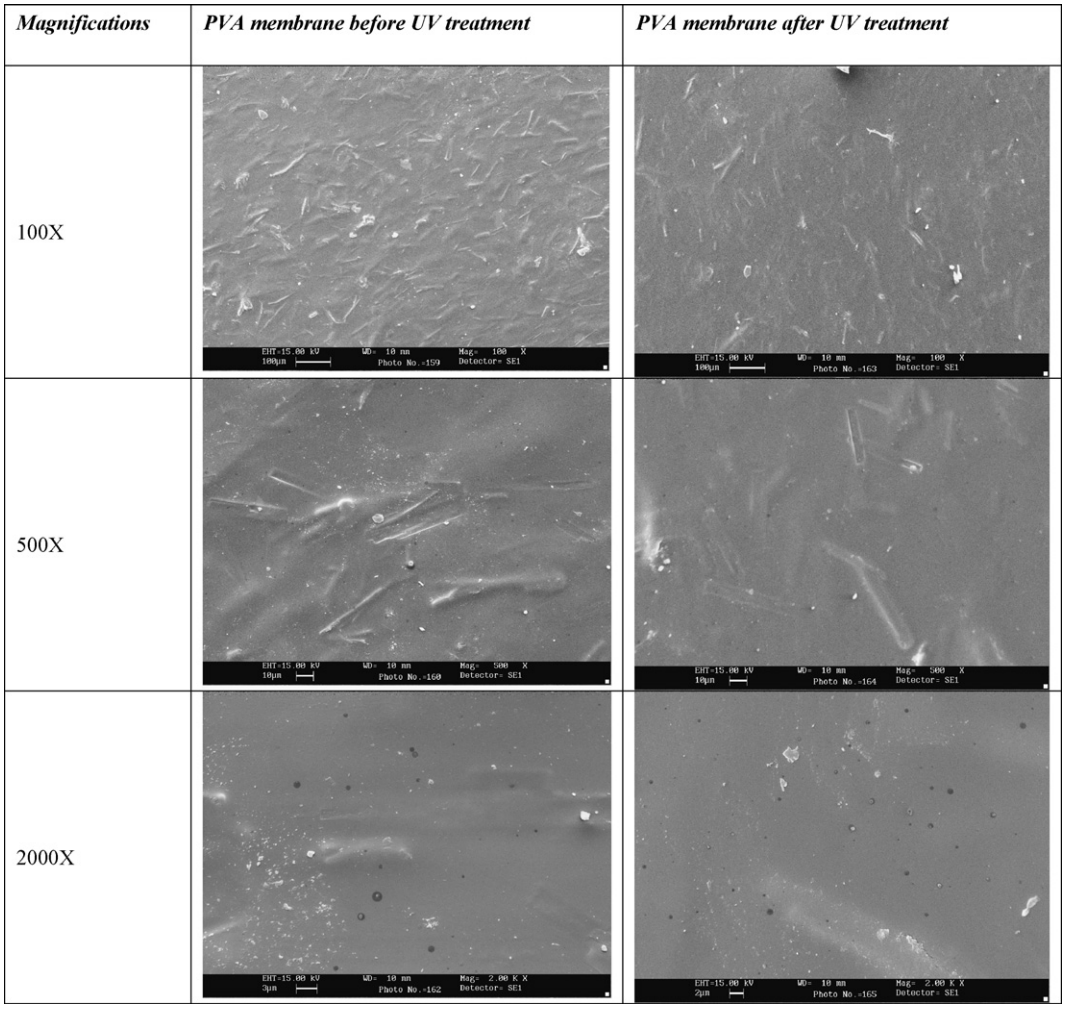


Fig. 3. SEM study of PVA membranes before and after UV treatment (magnifications: 100, 500 and 2000×).

and roughness and is smoother than the surfaces of the membrane before UV treatment (as shown in Fig. 3). The membrane with smoother surfaces has greater antifouling capability [23] for better quality and longevity.

### 3.4. Immobilization of GOD in PVA membrane

GOD of different concentrations was immobilized in PVA membranes and optimum activity was obtained when 2000 units/ml concentration was used. There after there was no further significant increase in activity. Hence 2000 units/ml concentration of GOD was used for further study.

Km for free and immobilized GOD was studied and was found to be 76 and 119 mM respectively. No drastic changes in Km on immobilization suggest any major alteration in its conformation.

### 3.5. Calibration of DO probe using GOD–PVA membranes

Calibration of DO electrode response with the standard concentration (0.9, 4.5, 22.5, 90, 225, and 450 mg/dl) of glucose was carried using GOD–PVA membrane. Linear fit ($Y = 0.1512 + 0.52X$) with a slope of 0.52 ($R^2 = 0.989$; S.D. = 1.9214) was estimated between 0.9 and 225 mg/dl of glucose.

### 3.6. Detection range, detection limit and response time

From the linear fit of calibration of DO electrode in response to standard concentration of glucose, the detection range of biosensor was estimated between 0.9 and 225 mg/dl. The detection ranges were comparable to the commercial glucose biosensor available in the market [15]. Standard deviation of the noise was $0.011 \times 10^{-2}$ ppm, twice of this inferred the detection limit 0.6 mg/dl of the biosensor.

The enzymatic reaction using immobilized enzyme was observed for 30 min and the readings were acquired after 2 min of interval in terms of change in depletion of dissolved oxygen. It was observed that maximum dissolved oxygen was consumed in first reading than the subsequent readings therefore 2 min was considered as response time of the biosensor.

### 3.7. Reusability, reproducibility and stability of the GOD–PVA membrane

The reusability of the four GOD–PVA membranes in response to glucose is shown in Fig. 4. Membranes were reused for 32 reactions without significant loss of activity.

The low relative standard deviations $2.8 \times 10^{-2}$ (mean = $46.2 \times 10^{-2}$ ppm, when $n = 6$) in the response of immobilized enzyme for 90 mg/dl glucose demonstrated the high reproducibility of analysis. Additionally, a very low relative standard deviation $3.1 \times 10^{-2}$ (mean = $46.78 \times 10^{-2}$ ppm) of three different experiments carried; using the same condition further demonstrated the result.

As shown in Fig. 5 the immobilized GOD–PVA membranes were stable for 30 days of investigation with retention of

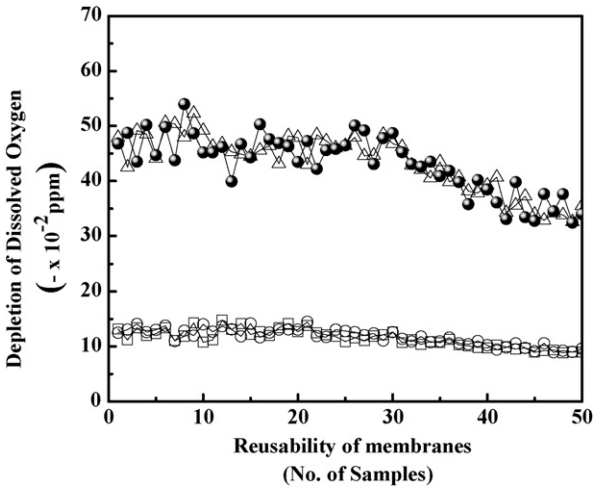


Fig. 4. Reusability of the GOD–PVA membrane (four GOD–PVA membranes were studied against glucose concentrations: (□) 22.5 mg/dl, (◯) 22.5 mg/dl, (△) 90 mg/dl and (●) 90 mg/dl).

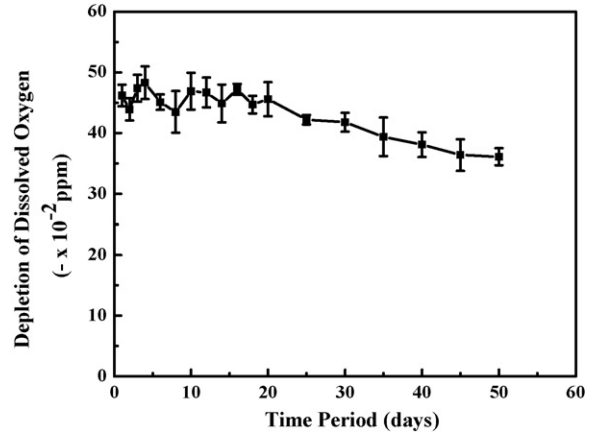


Fig. 5. Stability of the GOD–PVA membrane (a concentration of 90 mg/dl glucose was used for the study).

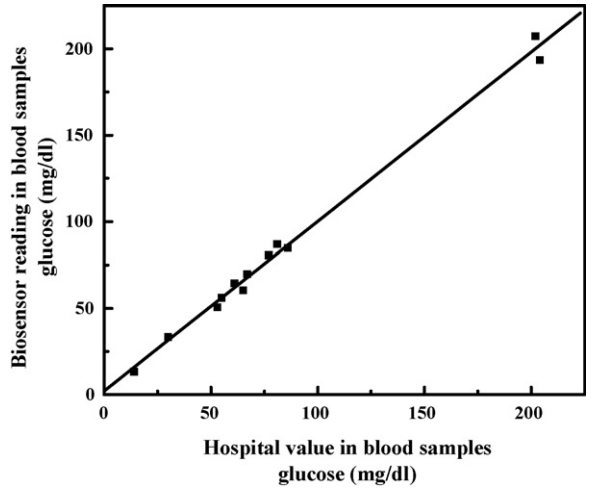


Fig. 6. Correlation of the biosensor reading (glucose mg/dl) with the BARC hospital value in blood sample. Linear fit ($Y = 2.2 + 0.979X$) and correlation ($R^2 = 0.992$).

90% activity, when stored at 4 °C and subsequently response decreased.

### 3.8. Detection of glucose in blood samples

Biosensor was also used to detect glucose in blood sample (from the BARC hospital). Biosensor readings were compared with the values obtained from the hospital. The correlation and straight-line fit plot between the two results was shown in Fig. 6. The straight-line fit yielded slope of 0.979 ($R^2 = 0.992$), which demonstrates the feasibility and a good correlation of the GOD–PVA membrane response with the existing analysis method. Blood consists of many other biological components, which did not interfere with the response of GOD–PVA membrane.

## 4. Conclusions

An alternate method to prepare the polyvinyl alcohol membrane for biosensor application using high and low DOP of PVA, acetone, benzoic acid and cross-linking by UV treatment is described. Swelling index study indicates that the membrane prepared with 12% low DOP and 8% high DOP of PVA, 2% BA, dissolved in buffer containing 20% acetone and cross-linked with the UV treatment, was having lower swelling index and suitable for immobilization. FTIR study showed appearance of strong band at ~2337 $cm^{-1}$ when UV was used for cross-linking in the presence of the benzoic acid. SEM study revealed the smoother quality of the membrane after UV treatment. Glucose oxidase enzyme was immobilized and GOD–PVA membrane was associated with the dissolved oxygen probe for biosensor reading. A wide detection range, 0.9–225 mg/dl was estimated and membrane was reused for 32 reactions without significant loss of activity. Membranes were stored for 30 days (~90% activity) at 4 °C. Use of GOD–PVA membranes were also demonstrated with real blood samples.

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