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Development of sensitive automated pH meter for real-time biosensor applications

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A computer controlled pH meter was developed using simple op-amp circuits. Analog to digital converter (ADC) card was used to acquire the output potential from the op-amp. Software based signal averaging helped in reduction of electrical noise. The instrument was calibrated against pH and ammonia selective electrodes where it followed Nernstian behaviour and was used in the development of a simple biosensor for urea. The enzyme urease was immobilized in polyvinyl alcohol - polyacrylamide composite polymer gel. The enzyme membranes were used in conjugation with the ammonia selective electrode connected to the fabricated instrument. The studies with this biosensor assembly yielded linear response behaviour with a response time of 180 sec.

1 Introduction

With increasing environmental pollution levels and resulting health concerns, demand for precise and rapid measurements of pollutants are growing [1]. Clinical diagnostics [2] and military concerns over security threats from biological warfare agents [3] have also produced the necessity of determination of analytes with high specificity. Agriculture, food processing, healthcare and-pharmaceutical industries need real-time and *in-situ* sample analysis [4]. Biosensors have emerged as the analytical devices that could perform analysis of samples in real-time environments. Being sensitive, reproducible and sometimes reusable, they hold promise to the market requirements. They are self-contained integrated devices capable of providing specific quantitative or semi-quantitative analytical information using a biological recognition element, which is in direct spatial contact with a transduction element [4-5]. The biological recognition element, e.g. enzymes, antibodies, microbial cells, DNA or RNA, animal or plant tissue etc., in conjugation with a transducer (e.g. optical, potentiometric, amperometric, acoustic, thermal and electrochemical etc.) can trigger physico-chemical changes upon interaction with the analyte under concern [4]. The resulting signal, e.g. current, potential or frequency etc., can be acquired using data acquisition and processing system.

Because of diffusional constraints it is essential that these biological recognition elements should be in close contact with the transducer. This can be achieved by immobilizing the biomaterials in synthetic or natural polymer. Immobilization not only helps in forming the required close proximity between the biomaterial and the transducer, but also helps in stabilizing it for reuse. Biomaterials can be immobilized either through adsorption, entrapment, covalent binding, cross-linking or a combination of all these methods [6-7]. A number of techniques have been developed in our laboratory for the immobilization of viable and non-viable cells as well as cell-enzyme conjugates [6-10]. Selection of a technique and/or support would depend on the nature of the biomaterial, substrate and configuration of the transducer used.

Microprocessor or micro controller based cheap laboratory devices are more common in the development of biosensors. But, a computer-controlled device supports automated analysis and is convenient for discrete analysis, where it prevents human error, improves accuracy and precision. Automation permits on-line monitoring in industrial and clinical processes, where information is needed in quasi real-time.

In the present study we have demonstrated the design, calibration and operation of a simple computer controlled pH meter that may find use in potentiometric biosensor systems especially for urea determination. A urea biosensor works on the principle of enzyme-catalyzed reaction of urease on its substrate urea. The reaction liberates ammonia that causes an alteration in the potential output from the transducer - NH_3 selective electrode.

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2 Experimental details

Chemicals and reagents

The standard buffer tablets were purchased from Qualigens fine chemicals, Mumbai. Polyvinyl alcohol (PVA) with degree of polymerization 1700 - 1800 and degree of hydrolysis between 98 - 99 mole percent was obtained from Loba Chemie, Mumbai. Urease (E.C. 3.5.1.5.) tablets were from Sigma Chemical Co. Acrylamide (extra pure), N,N'-methylenebisacrylamide (Bis), N,N,N',N-tetramethylethylenediamine (TEMED) and ammonium persulphate (APS) were procured from SISCO Research Ltd.,. Mumbai. All other chemicals used in the experiments were of high purity and analytical reagent grade. The reagents were prepared in double distilled water. The pH of the solutions was adjusted by addition of dilute acid or alkali while keeping the ionic strength constant with addition of KCl.

Electronic assembly and software for interfacing

An electronic circuit was developed using a high input impedance, ultra low offset voltage, low bias current op-amp LF 356 [11]. Due to the high impedance characteristics of the pH and ammonia selective electrodes a voltage follower was used at the first stage. As the signal was quite feeble to achieve a high sensitivity, the potential was further amplified ten fold by an op-amp inverter at the second stage. Data acquisition was automated by connecting the amplified output to a 12 - bit analog to digital conversion (ADC) card (PCL –207 ISA card, Dynalog India Ltd.). The electronic assembly with electrode connectors was placed in a grounded aluminium box as a further noise-suppression device. Theoretically, the ADC card has a resolution of 2.44 mV for a full-scale voltage of 10 V (± 5 V). Software based averaging of the signal was mandatory, as the circuit did not have any inbuilt noise filters. The program for interfacing the ADC card and the signal processing modules was developed in our laboratory using C++ language. The program acquired the signal at 30 kHz scan rate, averaged and translated one-averaged potential readings per second.

Calibration of instrument

The instrument was calibrated with respect to a commercially available analog pH meter (Model LI 127, Elico Ltd., Mumbai). Solutions having different pH values were placed in a beaker with a pH electrode (Toshniwal Brothers, Mumbai) immersed in it. Potential of the solutions was recorded using analog meter as well as the instrument under development.

For calibration of the instrument with ammonia, 20 ml solution of NH_4Cl to different concentrations was placed in a 50 ml beaker. Ammonia selective electrode (Orion Research Inc.) was immersed in this mixture, while avoiding trapping of air bubbles on the surface of the electrode. Data acquisition was initiated by adding 0.5 ml of 5 M NaOH in the solution.

Immobilization of urease

Urease was entrapped in the composite polymer membrane of PVA-polyacrylamide by free radical based polymerization. PVA (0.5 g), acrylamide (0.5 g) and Bis (50 mg) were added to 4 ml of water. The mixture was heated to dissolve the monomers and then brought to room temperature. 1000 units of urease dissolved in 1 ml of buffer were mixed to it. Free radical polymerization was initiated by addition of APS (50 mg) and TEMED (100 µL) and the mixture was filled between two glass plates separated by a polyethylene spacer of thickness 500 µm. Polymerization was performed at room temperature for 1 h.

The resulting gel measured 60 cm^2 in area. To evaluate the enzyme activity, membrane of size 1 cm^2 was cut and incubated in buffer containing 50 mM urea for 1 min. 20 µl aliquot was immediately withdrawn and mixed to phenol - hypochlorite reagent [12]. The colour produced was monitored spectro-photometrically at 625 nm. All the enzymatic reactions and sensor measurements were carried out in Tris -HNO_3 buffer (5 mM, pH 7), unless mentioned.

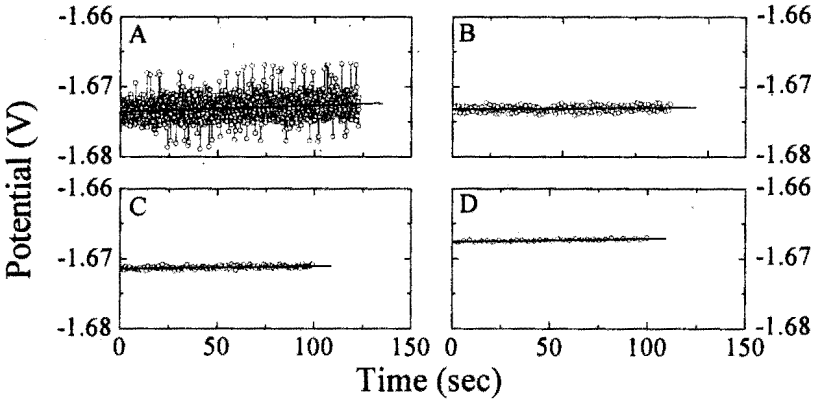

Fig. 1: Standardization of signal averaging period for the ADC card in standard buffer of pH 4. [A] Averaging time of 100 m sec (SD 17.1 mV); [B] 500 m sec (SD 4.45 mV); [C] 1000 m sec (SD 2.19 mV) and [D] 2000 m sec (SD 1.09 mV).

Sensor measurements

The enzyme membranes were stored at 4 °C and brought to room temperature prior to use. The membrane of area 1 × 2 cm^2 was cut and tied on the tip of the ammonia selective electrode with the help of O-ring. This was placed in a 50 ml capacity beaker containing 20 ml of buffer. The potential was recorded continuously and upon reaching the baseline (152.3 mV in this case), urea solution was injected in the medium with the help of clinical syringe.

3 Results and Discussions

Signal conditioning

An electrical noise in the circuit reduces the performance of the sensor not only by sacrificing the lower detection limit but also the sensitivity of the instrument. To overcome this problem a number of data points were averaged per unit time using software. It was observed that the sampling and averaging time had significant contributions to the signal quality (Fig. 1 A-D). For justification, a pH electrode was placed in the standard buffer of pH 4. For uniformity, all the potential measurements were carried in the same solution. Averaged data points were recorded with varying sampling time (100 m sec to 2 sec). Non-averaged signal had wide fluctuation from the mean. The standard deviation (SD) of data points from their mean position was as high as 17.1 mV for 100 m sec averaging period, whereas 1.09 mV for 2 sec of averaging. We have chosen-the averaging time of 1 sec (Fig .1C) as the representative of mean signal.

Calibration of the instrument with pH and ammonia selective electrodes

The fabricated pH meter, alongwith the software designed for proper signal conditioning was tested with respect to a commercially available analog pH meter. The straight lines drawn between the observed potentials of the pH solutions had nearly equal slopes for the first op-amp (follower circuit) of the instrument (58.32 mV / pH) and the commercial pH meter (58.45 mV / pH) (Fig. 2). The sign of the potential was inverted by second stage op-amp for ease in calculations in the basic pH range.

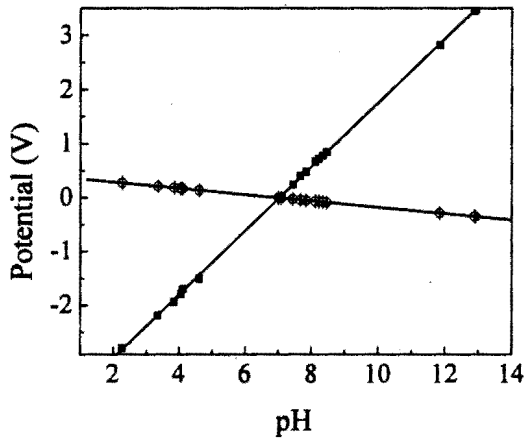

Fig. 2: Calibration of the computer controlled pH meter with respect to commercial analog pH meter. Straight-line fit for [○] commercial pH meter, (Y = 0.40913 - 0.05845 × X); [+] 1st op-amp output, (Y = 0.40839 - 0.05832 × X) and [■] 2nd op-amp output (Y = - 4.1544 + 0.59082 × X) of the fabricated instrument.

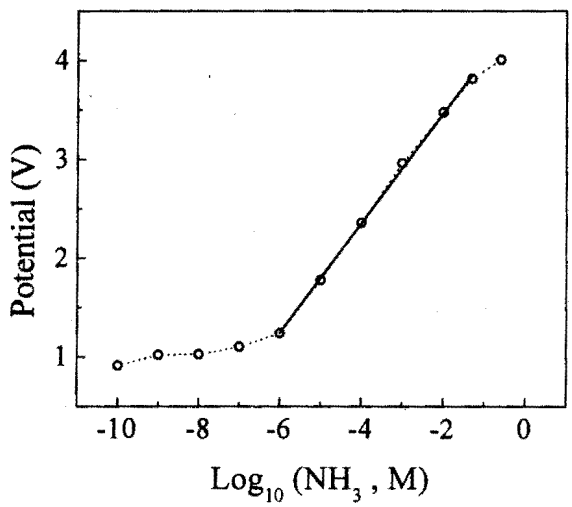

Fig. 3: Nernstian behaviour of pH meter with respect to different concentrations of NH_3 (dotted line). Straight-line fit at central portion of curve represents Y = 0.55533 × X + 4.57511, Chi^2 = 0.00121, R^2 = 0.99902.

The instrument was also calibrated with respect to ammonia solutions, as it was to be used alongwith an ammonia selective electrode. Upon addition of alkali to the ammonium chloride solution, the sudden change in pH liberated most of the ammonia, giving rise to an increased potential output from

the electrode, The plot of potential change versus ammonia concentration expressed in logarithmic scale obeyed Nernstian behaviour with the slope of 555.3 mV per decade of ammonia concentration with respect to second op-amp (Fig. 3).

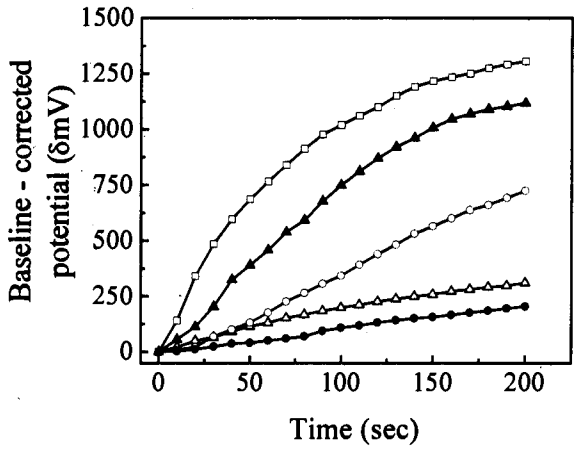

Fig. 4: The biosensor response curves for (•) 1 mM, (Δ) 10 mM, (○) 100 mM, (▲) 500 mM and (□) 1 M urea.

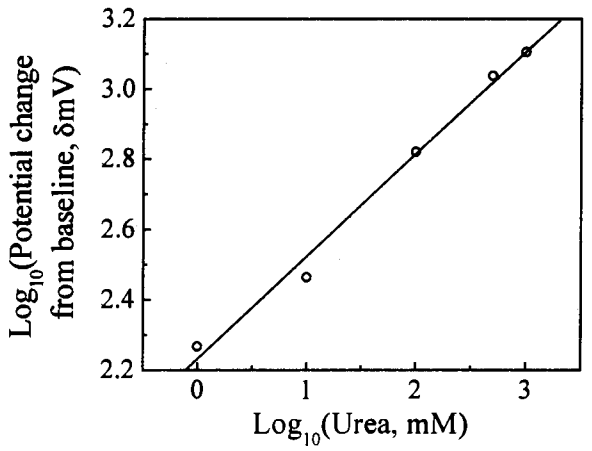

Fig. 5: The calibration curve for the biosensor at 180 sec response time (derived and plotted from Fig. 4). R^2 = 0.9951.

Sensor response

For biosensor measurements, the enzyme membrane was tied to the electrode tip and immersed in the buffer. Upon reaching baseline (stable electrode response), the enzyme- substrate reaction was initiated with addition of urea to the sensor assembly. The electrode potential increased due to formation of ammonia and saturated to values specific to particular concentrations of urea. The response of the sensor is shown in Fig 4. The concentration vs. potential curve for urea with 180-sec response time (Fig. 5) represented straight line with equation, Y = 2.23143 + 0.29165 × X. Where, Y is the baseline corrected potential and X the concentration of urea in mM (both in the logarithmic scale). The control

calibration curves obtained with enzyme-free membranes or buffer showed negligible response. Urea level ranges between 1 - 40 mM in human sera and 0 - 1 M in industrial effluent samples. The sensor had the working range of 1-1000 mM urea. This was broad enough to analyze the clinical as well as environmental samples.

4 Summary

A potentiometric system follows Nernstian equation with usual slope in the range of 55 to 58 mV per decade of analyte [13]. The developed instrument obeyed this equation. As a demonstration, we used the equipment in devising a simple biosensor for urea. The biosensor showed better sensitivity and wide range of detection. The calibration curve for the sensor showed linear behaviour with both the axis in the logarithmic scale. With online data acquisition capability and permissible modifications in the software, the instrument will be very useful in the further development of potentiometric biosensors.

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