

Langmuir–Blodgett Films of Polyaniline for Efficient Detection of Uric Acid

Kashima Arora, Monika Tomar, and Vinay Gupta

Abstract—Langmuir–Blodgett (LB) films of polyaniline (PANI) grown onto ITO coated glass substrates were utilized for the fabrication of Uric acid biosensor for efficient detection of uric acid by immobilizing Uricase via EDC–NHS coupling. The modified electrodes were characterized by atomic force microscopy (AFM). The response characteristics after immobilization of uricase were studied using cyclic voltammetry and electrochemical impedance spectroscopy techniques. The uricase/PANI/ITO/glass bioelectrode studied by CV and EIS techniques revealed detection of uric acid in a wide range of 0.05 mM to 1.0 mM, covering the physiological range in blood. A low Michaelis–Menten constant (K_m) of 0.21 mM indicates the higher affinity of immobilized Uricase towards its analyte (uric acid). The fabricated uric acid biosensor based on PANI LB films exhibits excellent sensitivity of 0.21 mA/mM with a response time of 4 s, good reproducibility, long shelf life (8 weeks) and high selectivity.

Keywords—Uric acid; biosensor; PANI; Langmuir Blodgett films deposition.

I. INTRODUCTION

BIOMOLECULAR electronics can be defined as science investigating the process in which electrons and electron currents of biomolecules are involved. It utilizes biomolecules or their complexes for application as modern electronic devices e. g. biosensors. These interesting biomolecular devices have been shown to have applications in clinical diagnostics, environmental monitoring, food freshness and bioprocess monitoring. Rapid growth in the development of new materials and improvements in sensing techniques have led to the evolution of advanced biosensors [1, 2]. In this context, conducting polymers such as polypyrroles, polythiophenes and polyanilines have become the materials of choice for recent technological advances in biotechnology [3, 4]. To realize a high performance miniaturized biomolecular electronic device, one of the key issues is the fabrication of ultra-thin ordered structures as the performance of the device depends strongly on the molecular arrangement. The assembling of molecules, known as molecular engineering, can be accomplished in many ways among which Langmuir–Blodgett [5] and self-assembly [6, 7] technique have been considered as most promising. The advantages of the LB technique are that highly uniform mono or multi-molecular

films can be produced in which orientation and packing can be controlled by applied surface pressure. Additionally, different components can be included in the molecular film in a predefined ratio. Thus advantage of controlling the geometrical arrangements and thickness of molecular films highlight this technique as an important method for the development of biosensors having short response time, good sensitivity, stability and increased dynamic range [8, 9].

Uric acid is an end product of purine metabolism in human body, a number of diseases and pathological disorders are related to its high concentration in body fluids (e.g. serum and urine), such as gout, arthritis, kidney disease, cardiovascular disease, and neurological diseases. The physiological normal level of uric acid in human blood is between 0.13 and 0.46 mM [10]. Consequently, uric acid determination is of paramount importance in the diagnosis of the diseases caused by disorder of purine biosynthesis and catabolism. □

In the present manuscript, we report results of studies relating to the preparation and characterization of uricase immobilized polyaniline (PANI) Langmuir–Blodgett films for detection of uric acid.

II. EXPERIMENT

For LB monolayer formation, spreading solution of polyaniline (PANI) mixed with stearic acid (SA) (1:1) was prepared and ultrasonicated for about 2 h. Monolayer deposition was carried out in an LB trough. An optimized amount (100 μ l) of the prepared solution was spread onto water subphase containing 4×10^{-4} M CdCl_2 . CdCl_2 was added to the subphase to obtain the desired degree of control over the monolayer behavior and successful deposition of multilayers as addition of these divalent ions reduces repulsion between the adjacent ionized groups of SA onto the water surface and making the monolayer more stable. The molecules present in a monolayer were compressed by a movable barrier to record the surface pressure–area isotherm. Fig. 1 shows the pressure versus mean molecular area isotherm of the prepared mixed monolayer on subphase. The value of pressure at which the quasi-solid behaviour of monolayer appeared was found to be 50 mN/m at subphase temperature of 293 K. Observed limiting mean molecular area of $\sim 33 \text{ \AA}^2$ in the present investigation confirms the formation of uniform mixed monolayer of polyaniline and stearic acid and is in agreement with the fact that for the formation of uniform mixed monolayer of PANI and SA the mean molecular area should

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be the sum of mean molecular area for SA and for PANI. At optimized pressure (50mN/m) and temperature (293K), 15

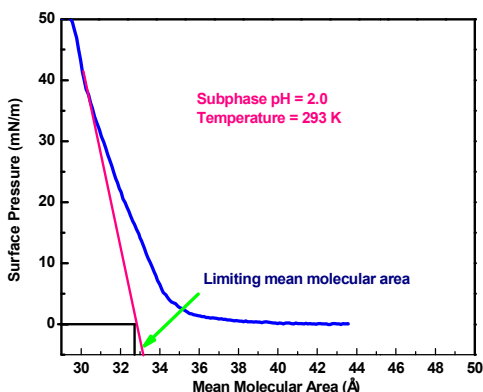
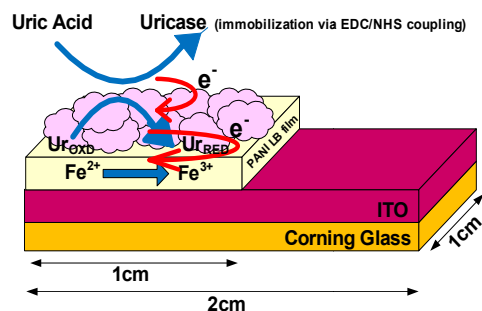


Fig. 1 Isotherm of polyaniline and stearic acid monolayer on de-ionized water containing $4 \times 10^{-4} \text{M CdCl}_2$

monolayers were transferred onto ITO coated substrates by vertical dipping at a speed of 10 mm/min.

At optimized pressure (50 mN/m) and temperature (293 K), 15 monolayers were transferred onto ITO coated substrates by vertical dipping at a speed of 10 mm/min.

For immobilization of uricase, the PANI/ITO/glass LB film electrode was incubated in the 1 mg/ml solution of uricase containing 0.2 M EDC and 0.05 M NHS for 4 h followed by washing with PBS. Fig. 2 shows the schematic of the fabricated bioelectrode Uricase/Pani/ITO/glass. The sensing response of the prepared uricase/PANI/ITO/glass LB film bioelectrode towards uric acid was studied using cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS). The film thickness was determined using a surface profiler (Dektak 150A). The surface morphology of the prepared uricase/PANI/ITO/glass bioelectrode was investigated using atomic force microscopy (AFM) (Veeco D1CP2).



NOTE:
 Ur_{oxd} : Uricase Oxidized
 Ur_{red} : Uricase Reduced

Fig. 2 Schematic of the prepared uricase/PANI/ITO/glass bioelectrode

III. RESULTS AND DISCUSSION

The surface morphology of the deposited PANI LB films before and after the immobilization of Uricase is investigated using atomic force microscopy (AFM) technique shown in Fig. 3.

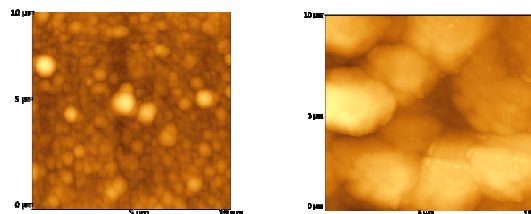


Fig. 3 AFM images of the (a) PANI/ITO/glass electrode (b) Uricase/PANI/ITO/glass bioelectrode

AFM image of the LB thin film shows rough surface morphology with uniformly distributed grains which is beneficial for efficient loading of enzyme on its surface. Upon immobilization of enzyme, globular structure of uricase molecules was formed.

CV curves were obtained for ITO/glass, PANI/ITO/glass and uricase/ PANI/ITO/glass electrodes. It may be noted from Fig. 4 that at a potential of 0.36 V and 0.14 V well defined oxidation and reduction peaks were observed for ITO/glass electrode. The potential of these oxidation and reduction peaks reduces to 0.28 V and 0.11 V for PANI LB film indicating better conductivity and good electrochemical properties of the prepared LB film of PANI. The peak oxidation current is found to decrease considerably on immobilization of uricase on the surface of PANI/ITO/glass electrode due to the non-conducting behavior and macromolecular structure of uricase.

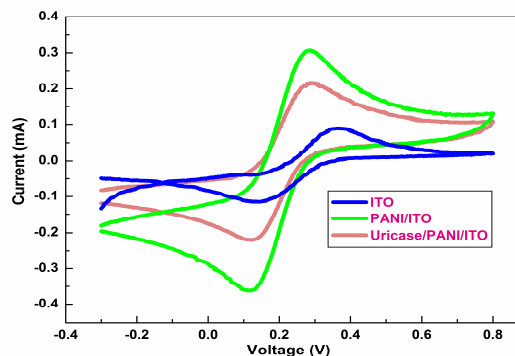


Fig. 4 Cyclic voltammetry response of ITO/glass electrode, PANI/ITO/glass and uricase/PANI/ITO/glass electrodes bioelectrode

The sensing response of the prepared biosensor (uricase/PANI/ITO/glass bioelectrode) was studied over the concentration range 0.05–1.0 mM of uric acid in mediated PBS (pH 7.0) solution. Concentration of uric acid (0.05–1.0 mM) is chosen so that it lies well beyond the physiological range (0.13–0.46 mM). It was observed that the peak oxidation current increases continuously with an increase in

uric acid concentration as shown in Fig. 5. The continuous rise in the oxidation current with increasing uric acid concentration is attributed to the release of more number of electrons in the oxidation of uric acid by uricase. The results indicate that the fabricated biosensor exhibit a good linearity in the peak oxidation current over a wide range of uric acid concentration (0.05–1.0 mM) (Fig. 5). The sensitivity of the uricase/PANI/ITO/glass bioelectrode was found to be $0.21 \text{ mA}(\text{mM})^{-1}$ in the range of 0.05–1.0 mM which is better than the one reported by other workers for uric acid biosensors [11, 12].

Electrochemical impedance analysis was performed with Gamry potentiostat/Galvanostat Ref.600 instrument, using a three-electrode cell with PANI/ITO/glass LB film as working electrode, platinum foil as counter and Ag/AgCl as reference electrode in PBS solution (50 mM, pH 7.0, 150 mM NaCl) containing 5 mM $[\text{Fe}(\text{CN})_6]^{3-/4-}$ in the frequency range of 100 KHz to 10 mHz. Fig. 4 shows EIS curves of (i) ITO/glass, (ii) PANI/ITO/glass LB film and (iii) Uricase/PANI/ITO/glass LB film electrodes, respectively. The decrease in charge transfer resistance (R_{ct}), from 425 Ω (ITO) to 222 Ω (PANI/ITO/glass) indicates conducting behaviour of PANI films as compared to ITO/glass. Further, enhancement in R_{ct} to 300 Ω for Uricase/PANI/ITO/glass indicates successful immobilization of non-conducting macromolecules of Uricase. Similar results are confirmed by cyclic voltammetry technique as well.

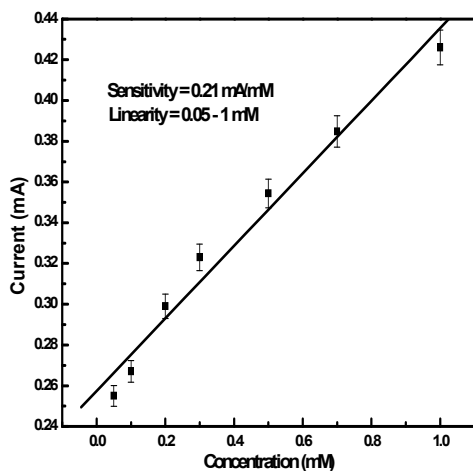


Fig. 5 Amperometric response of the fabricated uricase/PANI/ITO/glass bioelectrode with uric acid concentration

The prepared uricase/PANI/ITO/glass biosensor has a fast response time of about 4 seconds. The selectivity of uricase/PANI/ITO/glass bioelectrode is determined by measuring response current in CV analysis on addition of normal concentration of interferents in the human serum such as glucose (5.6 mM), cholesterol (5 mM), urea (1 mM), ascorbic acid (0.1mM) and dopamine (0.1mM) at normal concentration of uric acid (0.1mM) indicating maximum interference of 2.1%.

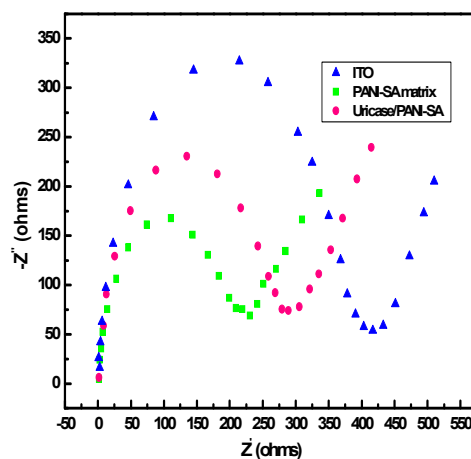


Fig. 6 EIS plots of (i) ITO/glass, (ii) PANI/ITO/glass LB film and (iii) uricase/PANI/ITO/glass bioelectrode

IV. CONCLUSION

An impedimetric conducting polymer LB film based biosensor was prepared for estimation of uric acid. The Uricase/PANI/ITO/glass LB film was found to detect uric acid in a wide range of 0.05- 1 mM indicating high loading of Uricase onto PANI/ITO/glass LB film with sensitivity of 0.21 mA/mM by cyclic voltammetry technique. EIS plot confirms better conductivity of PANI LB films as compared to ITO. Also, the presence of other interferents present in human serum has negligible effect on the performance of the biosensor. The prepared biosensor has a fast response time of 4 seconds and shelf life of 8 weeks.

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