Use of Polyfuran/Chitosan Composite Films Deposited by Atmospheric Pressure Plasma Glow Discharge as Glucose Sensors

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In this study, the properties of composites of polyfuran/chitosan (PFu/Ch) were electrochemically investigated as potential glucose sensors. Polyfuran (PFu) and polyfuran/chitosan composite films were coated onto indium thin oxide electrode by atmospheric pressure plasma glow discharge method.The prepared films were characterized by Fourier transform infrared spectroscopy and scanning electron microscopy analyses. Characteristic bands of both polyfuran and chitosan were observed in FTIR spectra of polyfuran/chitosan composite films. SEM images of the composite polyfuran/chitosan films showed more granular and porous surfaces than both of pure polyfuran and chitosan films. It was observed that though pure polyfuran films were not stable for glucose oxidase (GOD) immobilization. The polyfuran/chitosan composite films were quite stable and displayed good immobilization properties as enzyme sensors. Glucose oxidase enzyme electrodes were prepared onto the surface of polyfuran and polyfuran/chitosan composite films by physical adsorption method of glucose oxidize for comparison. Polyfuran/glucose oxidase and polyfuran/chitosan/glucose oxidase enzyme electrodes were utilized for glucose sensing using the amperometric method. The current response of polyfuran/chitosan/glucose oxidase electrode increased linearly with increasing glucose concentration, indicating a better glucose sensing for composite films.

Keywords: Atmospheric pressure plasma polymerization, Polyfuran, Chitosan, Biosensor.

INTRODUCTION

The methods used to synthesize and modify conducting polymer composites have varied from wet methods such as chemical and electrochemical to dry methods like vapour phase polymerization and plasma polymerization [1]. In chemical and electrochemical syntheses, the typical structure with alternate single and double bonds leads to rigid and insoluble polymers. Processing of the polymers resulting from chemical polymerization is therefore difficult [2,3].The problem can be resolved by using electropolymerization with deposition directly onto an electrode conductive substrate. Chemical and electrochemical syntheses are usually performed in batch processes, their most important advantage is in the prediction of the structure of molecules.

Recently the synthesis of thin polymer films by plasma polymerization has emerged as an interesting new approach [4,5]. Plasma polymerization is a solvent-free, room temperature process that can be used to rapidly deposit thin polymer films onto a wide variety of substrates [6]. The advantages of plasma polymerization include the environmental friendliness

of the solvent-free process, the deposition of ultra-thin films with thickness directly proportional to deposition time and the deposition of pinhole free films without dimensional changes associated with solvent evaporation [7]. Typically and till the last decade, most of the plasma deposition and coating techniques were carried out in low pressure plasma. The technical difficulties and non uniformity in high pressure plasmas are partially resolved in atmospheric pressure plasma systems. The advantages of atmospheric pressure plasmas are the elimination of vacuum chambers thus reducing the cost of the plasma system and the ease of modifying shape and size of the plasma systems in atmospheric pressure settings [8].

Using plasma methods in studies on the synthesis of conductive polymers are well documented [9] but studies on the composites are limited [10]. Among conducting polymers, polyaniline, polypyrrole and polythiophene have found a wide application area [11-13]. Due to its high oxidation potential, studies on polyfuran and its composites are very limited [14]. Compared with thiophene and pyrrole, the furan monomer is a low-cost raw material and has considerable applications in biosensor technology [15] and as a humidity sensor [16].

Plasma polymer films have microelectronics, functional polymer films and biopolymer applications. In recent years, the use of conductive polymers in the development of biosensors has drawn a lot of attention. Of particular importance is the compatibility of the films with biopolymers like chitosan and cellulose. Chitosan has been widely used in different areas such as water treatment systems and biosensors. The presence of conductive polymers [17] greatly improves the biosensors features.

Chitosan/polyaniline [18], chitosan/substituted polyaniline [19] and chitosan/polypyrrole composites obtained by chemical and electrochemical methods are modified as biosensors [18]. In these methods, the preparation of homogeneous electrodes is difficult, as heterogeneous structures can be formed onto the electrode surfaces. In electrochemical methods, conducting working electrodes such as platinum, gold and glassy carbon have been used to create films onto the desired electrode surface. In plasma methods, polymer films can be coated onto every material surfaces. Recent work has been reported on the electrochemical synthesis of polyfuran [20] copoly(furan/ pyrrole) [21], copoly(furan/2-methylfuran) [22] and polyfuran/ polythiophene bipolymer films [23]. In fact, a few scientists have synthesized polyfuran [24], polyfuran/poly(2-chloro-aniline) composites [25] and polyfuran/polyaniline [26] copolymers by chemical oxidative polymerization. However, there is no reported study on atmospheric plasma polymerization of polyfuran/chitosan (PFu/Ch) composites. The aim of the present study is to use atmospheric plasma polymerization to synthesize polyfuran/chitosan composites and therefore improve the properties of polyfuran. In our study, we used chitosan as the supporting biopolymer because of its favourable physico-chemical and biological properties [27,28].

Chitosan/polyfuran composite film is prepared by *in situ* polymerization of furan using atmospheric pressure plasma method. The glucose oxidase enzyme was attached onto the films in the presence of glutaraldehyde cross-linker. The enzyme activity was determined electrochemically by the increasing glucose concentration using amperometric method. The response current and biosensor parameters of polyfuran/ chitosan/glucose oxidase and chitosan/glucose oxidase enzyme electrodes were drawn using Lineweaver-Burk plots. Although the polyfuran was sythesized by *in situ* polymerization of furan using glow discharge atmospheric pressure plasma, the biosensor properties of polyfuran could not be investigated because of its weak film stability.

EXPERIMENTAL

Chitosan (Ch), acetonitrile, glutaraldehyde (25 %) and glucose oxidase (GOD) enzyme were obtained from Aldrich. Sodium phosphate mono hydrate and sodium hydroxide pellets were purchased from Riedel-de Haen and furan was obtained from Merck.

Atmospheric plasma graft polymerization of furan: The reactor used for plasma polymerization is obtained using 10 kHz 9600 Vp-p rf atmospheric pressure plasma. The atmospheric pressure glow discharge (Fig. 1) is obtained with a matching unit, argon flow. 0.2 mL of furan monomer was dropped on the indium thin oxide electrode. Glow discharge atmospheric pressure plasma [4] was applied for furan thin film graft polymerization. The deposition of polyfuran film on indium thin oxide electrode was obtained using argon gas at 10 kHz 9600 Vp-p rf and 11.65 L/min (liter per minute flow rate) for 7 min.

Atmospheric plasma polymerization of polyfuran/ chitosan (PFu/Ch) composite: 40 μL of chitosan (0.2 g/20 mL) solution in 2 wt % acetic acid was dropped on to the indium thin oxide electrode and dried at room temperature. Then 0.2 mL furan monomer was dropped on to the dried thin chitosan film. Polyfuran/chitosan composite film on indium thin oxide electrode was obtained using argon gas at 10 kHz 9600 Vp-p rf and 11.65 L/min (liter per minute flow rate) for 7 min.

Preparation of enzyme electrodes and amperometric measurements: NaH_2PO_4 (Riedel-de Haen), NaOH pellets (Riedel-de Haen) and distilled water were used to prepare the buffer solution. The enzyme electrodes of chitosan, polyfuran and polyfuran/chitosan films onto indium thin oxide were prepared by dropping 2.5 mg/mL glucose oxidase in 0.1 M phosphate buffer (pH = 6.9) on the composite film. Glutaral-dehyde crosslinker was used to arrest the glucose oxidase
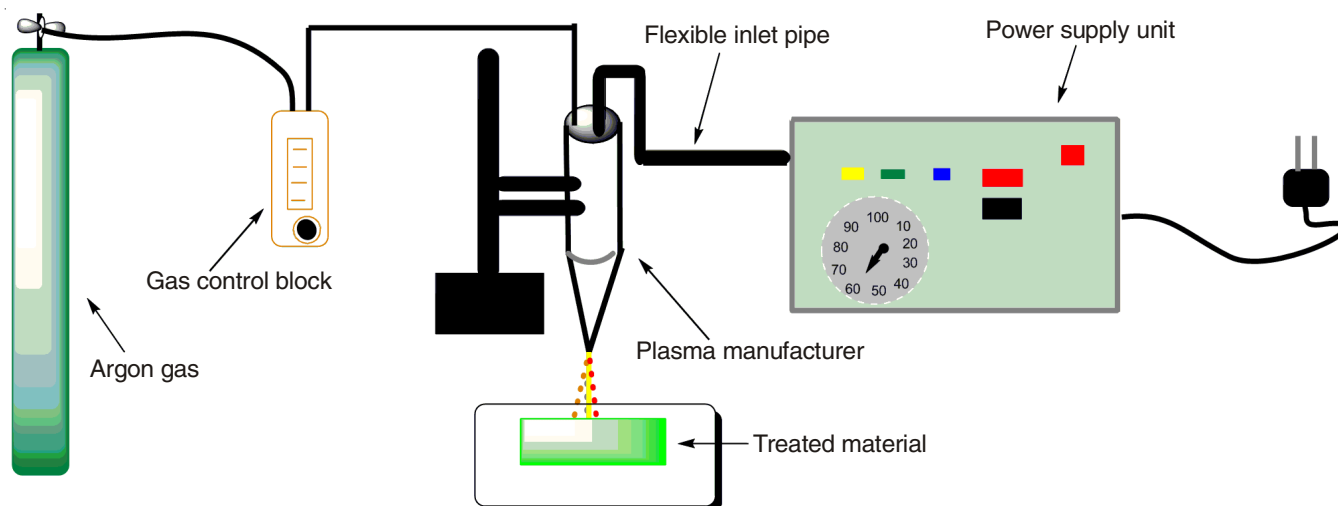

Fig. 1. Scheme of the atmospheric plasma polymerization system

enzyme onto the working electrode. The cross linked enzyme solution is prepared where the enzyme and cross linker ratio is 5 % (at pH 6.9, 2.5 mg/0.5 mL).

The response of the glucose oxidase enzyme electrodes to glucose was electrochemically investigated by the amperometric technique. Oxygen was introduced into electrochemical cell at a constant flow rate to obtain an oxygen-saturated solution. Oxygen flow over the solution was continued to keep it saturated during the measurements. When the background current stabilized to a constant value, a known amount of glucose solution was injected followed by 100 s of stirring. The steady state current was measured in 100 μL of 0.10 M phosphate buffered solution (pH 7.4) under a polarizing potential of 0.7 V [18]. Then, an increase in time-dependent current with the oxidation of hydrogen peroxide was recorded.

Characterization: FTIR was used to analyze the chemical structure of the plasma polymerization synthesized polyfuran (PFu), chitosan (Ch) and polyfuran/chitosan (PFu/Ch) composite. FTIR spectra of the films were recorded between 750 and 4000 cm^{-1} resolution on a Perkin Elmer System FTIR spectrometer. SEM images were taken on a scanning electron microscope model Tescan Vega II LSU. Electrochemical experiments were carried out using a Gamry 300 model potentiostat. Electrochemical measurements were performed in a conventional three-electrode cell at room temperature. The plasma polymer coated indium thin oxide electrode was used as working electrode. Ag/AgCl and Pt electrodes were used as the reference electrode and counter electrode, respectively.

RESULTS AND DISCUSSION

FTIR results: Figs. 2 and 3 show the comparison of FTIR spectra of polyfuran, chitosan and polyfuran/chitosan films onto indium thin oxide electrodes. The FTIR spectrum of atmospheric pressure plasma coated polyfuran thin film indicated four characteristic bands of 2,5-disubstituted furan units, corresponding to the C-H out-of-plane vibration at 798, the C-O-C streching vibration at 957 cm^{-1}, the C=C stretching vibration in the furan ring at 1604 cm^{-1} (Fig. 2) and the aromatic C-H streching at 3532 cm^{-1} (Fig. 3) [29]. The presence of these bands confirms the formation of polyfuran structure (Fig. 4a).

The major characteristic peak of chitosan at 3402 cm^{-1} is due to OH vibration (Fig. 3). The band is quite broad and displays the characteristic –NH$_2$ band for chitosan. Other observed bands were at 2892 cm^{-1} (C–H) (Fig. 3), 1682 cm^{-1} (C=O strech of carbonyl group), 1310 cm^{-1} (C–H stretch of methyl group), 1146 cm^{-1} (bridge O stretch) and 1030 cm^{-1} (C–O stretch) (Fig. 2). The band at 1586 cm^{-1} belongs to the N–H stretching of the primary amino groups [30,31].

FTIR spectra of polyfuran/chitosan composite film onto indium thin oxide electrode show characteristic bands of both chitosan and polyfuran. The O–H vibrations of chitosan (3402 cm^{-1}) band slightly increased (3412 cm^{-1}) in the polymer because of intramolecular hydrogen bonds between the polymer chains [19,32]. When comparing the spectra of chitosan and polyfuran/chitosan composites, we noticed that the composite shows characteristic bands of chitosan at 1030, 1310 and 1146 cm^{-1}.

The chemical bonds in polyfuran/chitosan were similar to those in the spectrum of chitosan. This result indicates that
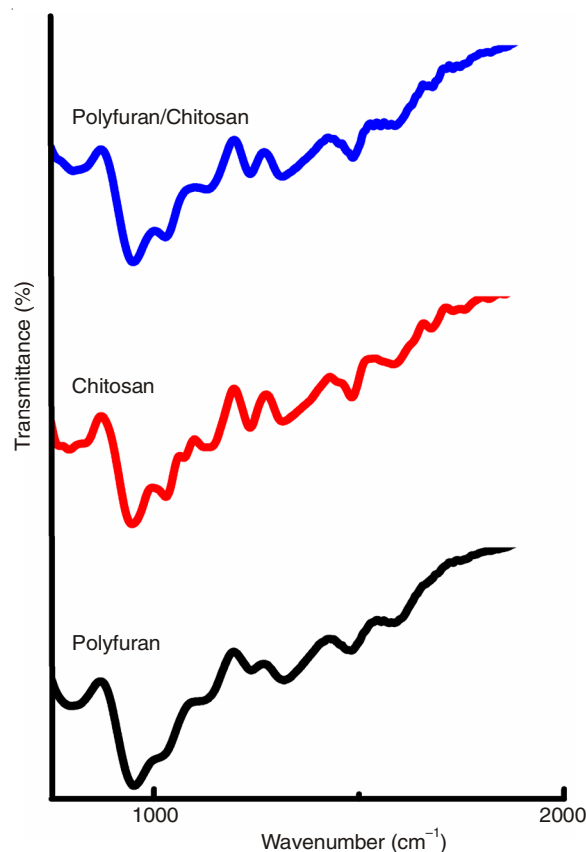

Fig. 2. FTIR spectrum of polyfuran (PFu), chitosan and polyfuran/chitosan composite between 750 and 2000 cm^{-1}
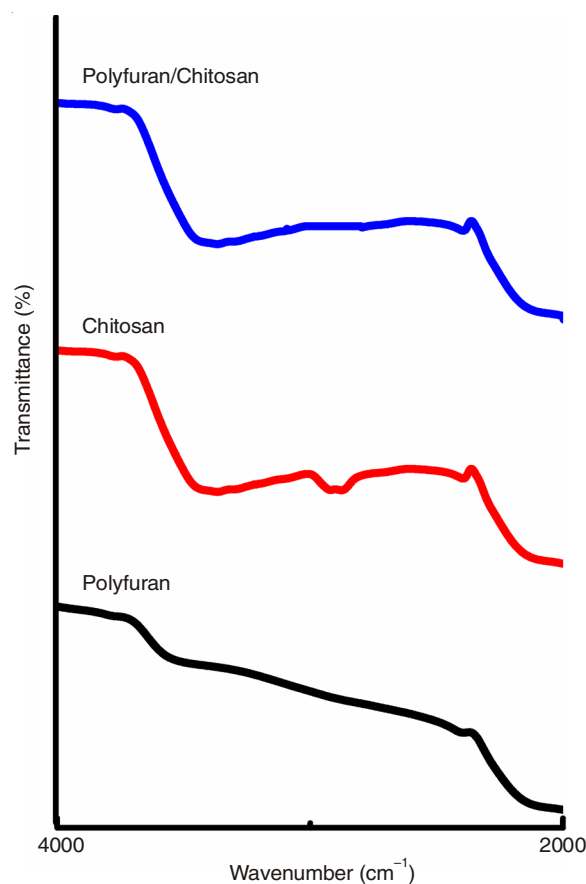

Fig. 3. FTIR spectrum of polyfuran, chitosan and polyfuran/chitosan composite between 2000 and 4000 cm^{-1}
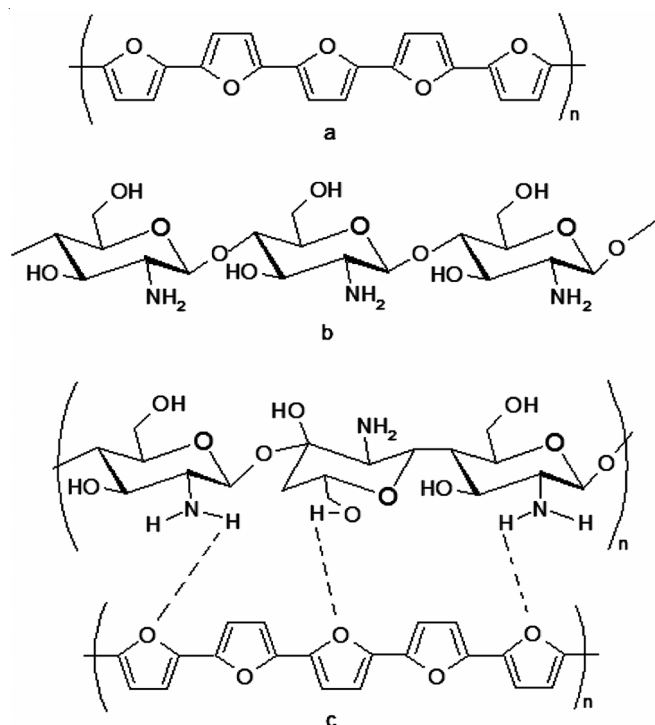
Fig. 4.   Structure of linear polyfuran(a), chitosan(b) and polyfuran/chitosan composite (c)

Fig. 6.   SEM images of polyfuran/chitosan (a), polyfuran/chitosan/glucose oxidase films (b)

Fig. 7. SEM images of polyfuran (a) and polyfuran/glucose oxidase films (b)

there was no significant difference between polyfuran/chitosan and chitosan and plasma modification and had no obvious impact on the principal chemical structure of the chitosan compound [33,34].

SEM results: Figs. 5-7 show scanning electron micrographs of chitosan (Fig. 5a), chitosan/glucose oxidase (Fig. 5b), polyfuran/chitosan (Fig. 6a), polyfuran/chitosan/glucose oxidase (Fig. 6b), polyfuran (Fig. 7a) and polyfuran/glucose oxidase (Fig. 7b) electrodes, respectively. As seen from Figs. (5a, 6a, 7a), all films have different surface morphologies. Chitosan film indicates a structure with layers [35] (Fig. 5a). Polyfuran surface morphology has a structure which includes some fibriller. The polyfuran/chitosan composite film has a granular structure.

SEM images of the enzyme electrodes were taken to confirm glucose oxidase immobilization onto polymer surface (Figs. 5b, 6b, 7b) [18,36]. Surface morphology is an important property when considering the immobilization of biomolecules [18,37]. Polyfuran/chitosan composite has a porous structure and presents the best morphological structure for the glucose oxidase molecule. For that reason, a remarkable change is
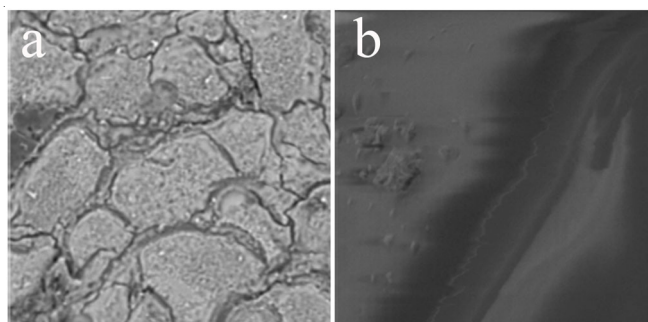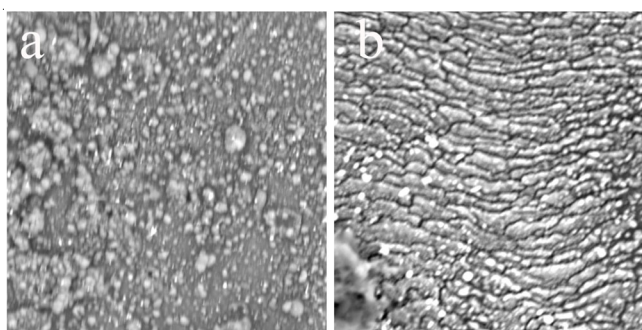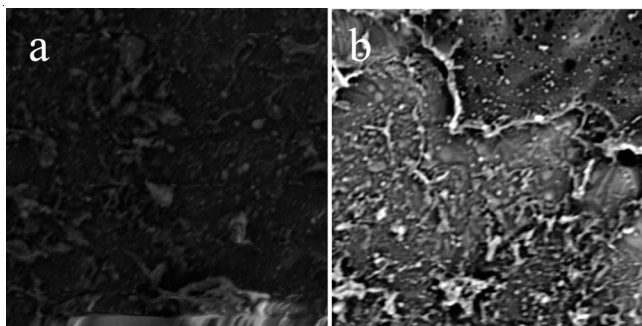

Fig. 5. SEM images of chitosan (a), chitosan/glucose oxidase films (b)

observed in the morphology of polyfuran/chitosan composite after glucose oxidase immobilization. As seen from SEM images, the biggest change was seen on the polyfuran/chitosan/glucose oxidase composite film electrode. Different surface morphologies after glucose oxidase immobilization have confirmed the attachment of the enzyme onto the electrode surface. As seen from SEM, the image of polyfuran/glucose oxidase enzyme electrode (Fig. 7b) is essentially not different than the surface morphology of polyfuran film without glucose oxidase. Moreover, the activity of polyfuran/glucose oxidase enzyme electrode could not be investigated, because the glucose oxidase immobilization onto polyfuran film indicated unstable behaviour. After combination of polyfuran with chitosan, a stable film could be obtained for enzyme activity measurements. Among all conducting polymers polyfuran has the least sensor application.

Amperometric determination of glucose and morphological results of biosensor electrodes: According to the Lineweaver-Burk equation, (eqn. 1), the Michaelis-Menten value which is an indication of the enzyme substrate kinetics, can be calculated using the linear relation between the mutual of the response current (I^{-1}) and the mutual of glucose concentration (C^{-1}).

$$\frac{1}{I} = \frac{K_m}{I_m} \times \frac{1}{C} + \frac{1}{I_m} \qquad (1)$$

The above straight line equation of the the form of $y = mx + n$ has a slope of $m = K_m/I_m$ and intercept of $n = 1/I_m$. As seen from the Lineweaver-Burk equation, the relation between the reciprocal of the response current and the reciprocal of glucose concentration is linear. Generally, K_m has been used to evaluate enzyme activity. As the enzyme loading increases, a bigger fraction of the glucose oxidase locates near

	TABLE-1 COMPARISON OF THE RESPONSE TIME, R^2, K_m FOR ENZYME, CHITOSAN/GLUCOSE OXIDASE, POLYFURAN/CHITOSAN/GLUCOSE OXIDASE BIOSENSOR ELECTRODES				
Sample	Response time (s)	R^2	Equation	K_m
Chitosan	100	0.974	y = 74.23x + 36292	0.00204
Polyfuran/chitosan	100	0.990	y = 255.65x + 41788	0.00612

the surface of the electrode, so as the average enzyme–substrate binding possibility increases, while the value of K_m decreases [24,38,39]. The equations of the response time and coefficient values of the modified enzyme electrodes are given in Table-1. The response current of chitosan/glucose oxidase enzyme electrode increased with increasing glucose concentration (Fig. 8). For the chitosan/glucose oxidase system, a linear calibration graph was obtained for current density *vs.* substrate concentration between 0.0001 and 0.0025 mM glucose. A linear relation was defined by eqn. 2:

$$y = 74.23x + 36292 \ (R^2 = 0.974) \tag{2}$$

where y is the sensor response in current density (A cm^{-2}) and x is the substrate concentration in mM.
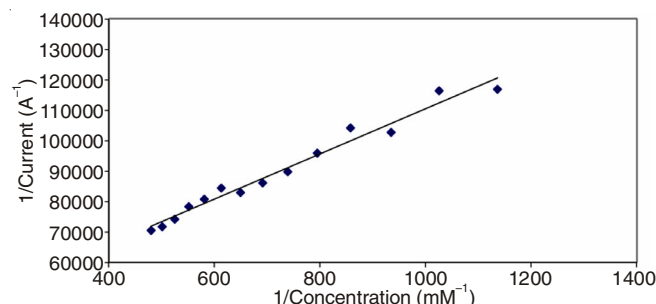

Fig. 8. Lineweaver–Burk plot for chitosan/glucose oxidase enzyme electrodes with glucose at 0.7 V in 0.10 M phosphate buffer solution (pH 6.9; 2.5 mg/mL glucose oxidase)

The polyfuran/chitosan/glucose oxidase composite biosensor has a linear range (Fig. 9) as defined by eqn. 3:

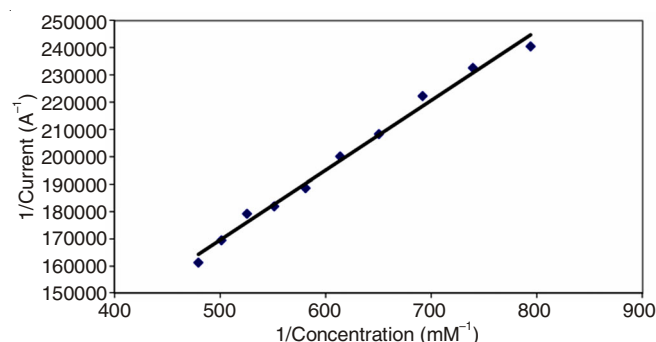$$y = 255.65x + 41788 \ (R^2 = 0.990) \tag{3}$$

Fig. 9. Lineweaver–Burk plot for polyfuran/chitosan/glucose oxidase enzyme electrodes with glucose at 0.7 V in 0.10 M phosphate buffer solution (pH 6.9; 2.5 mg/mL glucose oxidase)

Michaelis–Menten constant values K_m (gradient of the line) of the biosensors are found as 0.00204 and 0.00612 mM glucose. K_m values of chitosan and polyfuran/chitosan were lower than those on glucose oxidase absorbed chitosan-gold nanoparticles film [40] and PANI/chitosan-H$_2$SO$_4$ [18], indicating an obvious evidence of high sensitivity of the glucose

response [41-43] on the chitosan/glucose oxidase and polyfuran/chitosan/glucose oxidase electrodes.

Conclusion

Polyfuran and polyfuran/chitosan composite films were obtained by atmospheric pressure plasma polymerization onto indium thin oxide electrodes for glucose biosensor applications. The films were characterized using FTIR and SEM analyses. FTIR results have confirmed that there is an interaction between polyfuran and chitosan. SEM images with different surface morphologies after glucose oxidase immobilization have confirmed the attachment of the enzyme onto the electrode surface.

The deposition of the polyfuran/chitosan composite films in smooth continuous film is performed on indium thin oxide electrodes. The importance of the composite films is in the wide range of applications they can have. Enzyme electrodes, DNA biosensors, amperometric biosensors and in redox reaction are some examples of these applications.

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