Available online at www.sciencedirect.com
SCIENCE DIRECT
Talanta 67 (2005) 997–1006
Talanta
www.elsevier.com/locate/talanta

Studies on differential sensing of dopamine at the surface of chemically sensitized ormosil-modified electrodes

P.C. Pandey *, B.C. Upadhyay

Department of Applied Chemistry, Institute of Technology, Banaras Hindu University, Varanasi 221 005, India

Received 9 March 2005; received in revised form 15 April 2005; accepted 18 April 2005
Available online 31 May 2005

Abstract

We report herein the preparation of few chemically sensitized organically modified sol–gel glass (ormosil) films and sensing of dopamine at the surface of the modified electrodes derived from these films. The chemical sensitization in ormosil-modified electrodes is introduced by incorporating: (a) potassium ferricyanide and (b) either Nafion, or dibenzo-18-crown-6 or in situ generated Prussian blue from potassium ferricyanide. Electrochemical sensing of dopamine on the surfaces of these modified electrodes have been investigated and found that: (i) the presence of dibenzo-18-crown-6 facilitate the magnitude of dopamine sensing, (ii) conversion of potassium ferricyanide into Prussian blue also enhances the magnitude of dopamine sensing as compared to that of control and Nafion sensitized modified electrodes, (iii) both dibenzo-18-crown-6 and Nafion sensitized ormosil-modified electrodes are found selective to dopamine in the presence of ascorbic acid present under physiological concentration range. These finding again directed our attention to investigate the sensing of dopamine: (a) on dibenzo-18-crown-6 incorporated within Prussian blue sensitized modified electrode and (b) in the presence of varying concentrations of dibenzo-18-crown-6 in the Prussian blue modified electrodes. The investigations made on these lines again suggested the following: (1) increase in dibenzo-18-crown-6 concentrations in the modified electrode increases the magnitude of dopamine sensing upto an optimum concentration of macrocycle; (2) the detection limit of dopamine sensing goes down to 30 nM as compared to that of dibenzo-18-crown-6 incorporated with potassium ferricyanide which was found to the order of 100 nM. Investigations of the interference of ascorbic acid revealed that the presence of dibenzo-18-crown-6 introduces selectivity in dopamine sensing in the presence such common interfering analyte like ascorbic acid.

Keywords: Chemically sensitized ormosil film; Modified electrode; Dopamine sensing; Voltammetry

1. Introduction

The material chemistry originating through sol–gel process has increasingly gaining attentions of world scientists as such materials have wide practical applications in various directions. Organically modified sol–gel glasses (ORMOSILS) are one of the categories of such materials that provided wider applications to sensors technologist especially in electrochemistry [1,2] and are deriving from organically functionalized alkoxysilanes. Further encapsulation of electron transfer mediators having reversible redox electrochemistry within ormosil film is again potential requirement that might facilitate charge-transfer process within ormosil film required for probing chemical/biochemical interaction taking place within or outside the nano-structured domains of solid-state. One way to improve the electrochemistry of ormosil encapsulated mediators is the preparation of metal–ceramic composite that possibly lead to generate increased hopping sites of electron transfer within solid-state network. The other way for the same is to incorporate chemicals that affect the transport of ion as well as charge transport within solid-state matrix. Nafion and crown ethers fall amongst such chemicals; accordingly, it was proposed to study the electrochemistry of electron transfer mediators in the presence and absence of these chemicals whether they influence the charge transport within solid-state nano-geometry.

We have made investigations [3–5] on the electrochemistry of electron transfer mediators encapsulated within

* Corresponding author. Tel.: +91 542317745; fax: +91 542368174.
E-mail addresses: pcpandey@bhu.ac.in, p_c_pandey@satyam.net.in (P.C. Pandey).

0039-9140/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.talanta.2005.04.030

ormosil film derived modified electrodes and based on electrochemical measurements concluded the following: (1) incorporation of palladium within the nanoporous geometry of ormosil facilitate the charge transport with excellent redox electrochemistry of encapsulated mediator followed by introducing electrocatalytic power in ormosil-modified electrode; (2) incorporation of ruthenium derivatives also facilitate the charge transport within solid-state matrix as well as influence the electrocatalytic efficiency of the material. All these observations reveal the contribution of metal–ceramic composite to improve the electrochemistry and electrocatalytic properties of the modified electrodes. On the other hand, introduction of organic moieties having ion-exchange and ion-recognition site within ormosil matrix might alter the electrochemistry of ormosil-encapsulated mediators. Accordingly, it was aimed to investigate on these lines in the presence of Nafion and dibenzo-18-crown-6. We have observed that the ormosil film derived from 3-aminopropyltrimethoxysilane, 2-(3,4-epoxycyclohexyl)ethyltrimethoxysilane and phenyltrimethoxy silane in acidic medium retain the inherent property of these organic moieties and the resulting film is highly stable for practical applications. Potassium ferricyanide is also encapsulated together with such moieties that again generate excellent ormosil film for electroanalytical applications. Indeed we got interesting observations on the encapsulation of potassium ferricyanide/potassium ferrocyanide within the ormosil derived from 3-aminopropyltrimethoxysilane, 2-(3,4-epoxycyclohexyl)ethyl trimethoxy silane and phenyltrimethoxy silane in the presence of Nafion/crown ether which are reported in the present investigation. We further observed another interesting finding when tetrahydrofuran (THF) or cyclohexanone is allowed to interact with 3-aminopropyltrimethoxysilane in the presence of potassium ferricyanide. Such interaction resulted the formation of charge-transfer type complex and appear analogous to Prussian blue, however, THF result light blue colour whereas cyclohexanone result dark green colour. These coloured reaction products ultimately converted into transparent ormosil film in solid-state whereas control ormosil with encapsulated potassium ferricyanide without having interaction with THF/cyclohexanone resulted into light yellow colour. Results on visible photography are reported. The electrochemistry of potassium ferricyanide/ferrocyanide encapsulated within these chemically sensitized ormosil-modified electrode is reported. These modified electrodes are tested for the electrochemical sensing of dopamine.

Dopamine play crucial role in central nervous system and belongs to the group of catecholamines. Such chemical is a sympathetic neurotransmitter and plays an important role in the metabolism of sugars, lipids, etc. [6]. These chemicals are used in the treatment of acute congestive and renal failure [7] which concentration in urine samples of healthy animals without pheochromocytoma has been found between 100 and 400 μg/24 h^3. The variation of dopamine level in human physiological fluid has been reported in between 2 μM and 2 mM. It has now become an important analyte for analytical biochemists and accordingly development of methods for sensing dopamine has become an attractive requirement. The common instrumental techniques like HPLC have been widely used for the determination of dopamine [8–10]. Recent review summarizes various analytical methods such as HPLC, capillary electrophoresis techniques, and fluorescence methods for determining urinary catecholamines in healthy subjects based on five criteria, i.e., analytical range, limit of detection LOD, precision, application in real samples, and automation of the method) [8]. HPLC methods are more sensitive (LOD in the range of 1 nM) than capillary electrophoresis and fluorescence methods (LOD in the range of 10 nM). Such methods of catecholamine detection are often complicated and very expensive. The use of lipid films supported on a polymer has been frequently used for sensing dopamine. The stabilized lipid films have been used as detectors for the rapid repetitive analysis of this stimulant. The receptor molecules are incorporated in the mixture during preparation of the lipid membranes supported on the polymer. The incorporation of calixarene receptor into lipid membrane to enhance selectivity for dopamine sensing has been made [11–14] which was attributed to “molecular recognition”, involving cavity-shape fitting and hydrogen-bonding interactions, as common for resorcinol-derived calixarenes [15,16]. The presence of such receptors prevents the sensing of other catecholamines, adrenaline and other electroactive species. The electrodes coated with calixarene film have shown selective sensing of spiked dopamine in urine sample. A recent report described an optical spot test for rapid and selective detection of dopamine in human urine using stabilized in air dried lipid film [17].

Electrochemical sensors using modified electrodes have been widely used for the rapid detection of this stimulant. The electrochemical sensors for dopamine based on LB film [18], microfluidic system [19], biomemetic approach [20,21], sol–gel material based electrodes [22,23], enzymeless biosensor [24], bienzymatic system [25], enzyme amplification system [26], plant tissue [27–29] and voltammetric electrodes [30,31] have been reported. The use of Nafion membrane and Nafion coated tissue powder has been described to increase the selectivity of dopamine sensing [32,33]. The detection limits of dopamine sensing in several electrochemical systems were to the order of 5, 0.1, and 25 μM for adrenaline, dopamine, and ephedrine, respectively. The selectivity coefficients as proposed by Wang [34] and Wang and Chen [35] were found to be 11.5 for dopamine and 0.64 for ephedrine, if adrenaline is the primary species. Our contribution on dopamine sensing start with the use of polyphenol oxidase (tyrosinase) based electrode derived from palladium-linked ormosil [3]. Such modified electrode, although responded nicely, however, both sensitivity and selectivity of dopamine sensing was very poor required for practical applications. Apart from abundance literature available on dopamine sensing as described above, there still exists a need for real time selective analysis of dopamine based on non-enzymatic system that has been attempted in the

Table 1A
Composition of ormosil's precursors for making system-1, -2 and -3

System	A (μl)	B (μl)	C (μl)	D (μl)	E (μl)	F (μl)	G (μl)	H (μl)
1	70	10	5	–	–	60	240	5
2	70	10	5	–	5	60	235	5
3	70	10		5	–	60	240	5

A, 3-aminoproyltriethoxy silane; B, 2-(3,4-epoxycyclohexyl)ethyl trimethoxysilane; C, phenyltrimethoxy silane; D, dibenzo-18-Crown-6 (2 mg/ml) in phenyltrimethoxysilane; E, ethanolic solution of Nafion as supplied by Aldrich; F, aqueous solution of potassium ferricyanide (10 mM); G, distilled water and H, HCl (0.5N).

present investigation. Electrochemical oxidation of dopamine at the surface of chemically sensitized ormosil-modified electrodes as described above is reported in this communication. The systems provided deeper insight on the electrochemistry of dopamine oxidation as well as novel approach to introduce selectivity for sensitive sensing of dopamine.

2. Experimental

2.1. Materials

3-Aminopropyltrimethoxysilane, alcoholic Nafion solution, dibenzo-18-crown-6 and phenyltrimethoxysilane were obtained from Aldrich Chemicals Company. Dopamine was obtained from Sigma Chemicals Company. The aqueous solutions were prepared in triple-distilled water. All other chemicals employed were of analytical grade.

2.2. Construction of ormosil-modified electrodes

The modified electrodes were made on clean and dried indium tin oxide (ITO) electrode using 10 μl of homogenized ormosil's precursors solution of desired composition as given in Tables 1A and 1C. For easy presentation, the various compositions of the ormosil's precursors leading to different electrochemical properties of the subsequent modified electrodes could be designated as system-1 (control), system-2 (Nafion® sensitized); and system-3 (dibenzo-18-crown-6 sensitized). System-4 and -5 were also studied that could be made with Prussian blue obtained through chemical reaction between tetrahydrofuran/cyclohexanone, 3-aminopropyltrimethoxysilane and potassium ferricyanide. The composition of these reagents is given in Table 1B. When these reagents are mixed together, the yellow colour of potassium ferricyanide was converted into light blue with tetrahydrofuran and dark green with cyclohexanone under ambient conditions. The complete reaction product was used to make system-4 and system-5. These two systems differ from each other in absence (system-4) and the presence (system-5) of dibenzo-18-crown-6. The composition of ormosil's precursors is given in Table 1C. The system-5 could be again differentiated into 5a, 5b, 5c and 5d based on varying concentrations of dibenzo-18-crown-6 as shown in Table 1C. The ormosil's precursors as shown in Tables 1A and 1C were homogenized by stirring and 10 μl of each homogenized suspension was layered on cleaned and dried ITO electrode. The ormosil's film was allowed to dry for 5–7 h. The electrode was assembled in electrochemical cell and the electrochemistry of modified electrodes in each case was examined by cyclic voltammetry.

2.3. Electrochemical measurements

The electrochemical measurements were performed with a Solartron Electrochemical Interface (Solartron 1287

Table 1B
Conversion of potassium ferricyanide into Prussian blue

Reagent	3-Aminoproyltriethoxy silane (μl)	$K_3Fe(CN)_6$ (10 mM) (μl)	Colour of reaction product
Tetrahydrofuran (10 μl)	70	60	Light blue (R)
Cyclohexanone (5 μl)	70	60	Dark green (R)

R stands for total content of reaction product.

Table 1C
Composition of ormosil's precursors for making system-4, -5a, -5b, -5c and -5d

System	X	B (μl)	C (μl)	Y (μl)				G (μl)	H (μl)
				(i) 0 mg/ml	(ii) 0.5 mg/ml	(iii) 1.0 mg/ml	(iv) 2.0 mg/ml		
4	(R)	10	5	–	–	–	–	240	5
5a	(R)	10	5	5	–	–	–	–	5
5b	(R)	10	5	–	5	–	–	235	5
5c	(R)	10	5	–	–	5	–	235	5
5d	(R)	10	5	–	–	–	5	235	5

X, reaction product R (Prussian blue) as shown in Table 1B; Y, solution of dibenzo-18-crown-6 in tetrahydrofuran; B, 2-(3,4-epoxycyclohexyl)ethyl trimethoxysilane; C, phenyltrimethoxy silane; G, distilled water and H, HCl (0.5N).

Electrochemical Interface). An electrochemical cell made up of two pieces of Teflon® (6 cm × 6 cm × 1.8 cm) was used for the measurements. The upper piece of Teflon had arrangement of holding Ag/AgCl reference and platinum foil counter electrodes with 3 ml of working solution. The ormosil film made on ITO electrode was sandwiched between these two pieces of Teflon having the arrangement of gold connector for electrical connection. The cyclic voltammetry was studied between −0.2 and +0.6 V versus Ag/AgCl at desired scan rate. The amperometric measurements using the modified electrode were obtained at the desired operating potential vs. Ag/AgCl in the presence and absence of dopamine. The experiments were performed in phosphate buffer (0.1 M, pH 7.0) at 25 °C. Purging nitrogen for 15 min into the electrochemical cell provided insensitivities to any environmental interfering analyte including oxygen under working potential range in present experimental conditions.

3. Results and discussion

Our recent report describes on the contribution of ionophore-encapsulated ormosil's films useful for sensing hydrogen peroxide [36]. While proceeding ahead on the preparation of ormosil film using dibenzo-18-crown-6, we noticed the problem of its solubility in the macrocycle in ormosil's precursors. Fortunately dibenzo-18-crown-6 was little soluble into phenyltrimethoxysilane and the initial observations were satisfactory with system-3. However, we further noticed that the data on different concentrations of crown ether would be valuable to many electro analytical applications; accordingly, we attempted to increase the concentration of dibenzo-18-crown-6 in ormosil film using tetrahydrofuran as solvent. During such experimentation we observed that potassium ferricyanide get converted into Prussian blue in the presence of 3-aminopropyltrimethoxysilane and tetrahydrofuran or cyclohexanone. Such conversion again directed out attention to study the system derived from only Prussian blue in the ormosil film and further in the presence of varying concentrations of dibenzo-18-crown-6 together Prussian blue within the film. It should be noted that during such addition care was always taken to retain the inherent property of dibenzo-18-crown-6 within the film. Accordingly, two more systems system-4 (with Prussian blue only)] and system-5 (with Prussian blue and varying concentrations of dibenzo-18-crown-6) have been made as discussed in Section 2. After having these five system made, we intended to study the following: (1) the variation in electrochemistry of $K_3Fe(CN)_6$ in different systems if any; (2) to study the chemistry of Prussian blue formation; (3) to study interaction of potassium ferricyanide and dibenzo-18-crown-6 if any; (4) to study the dopamine sensing on these modified electrodes; (5) to study the effect of dibenzo-18-crown-6 on dopamine sensing, if any; (6) to study the selectivity of dopamine sensing. The findings on these lines are reported in following sections.

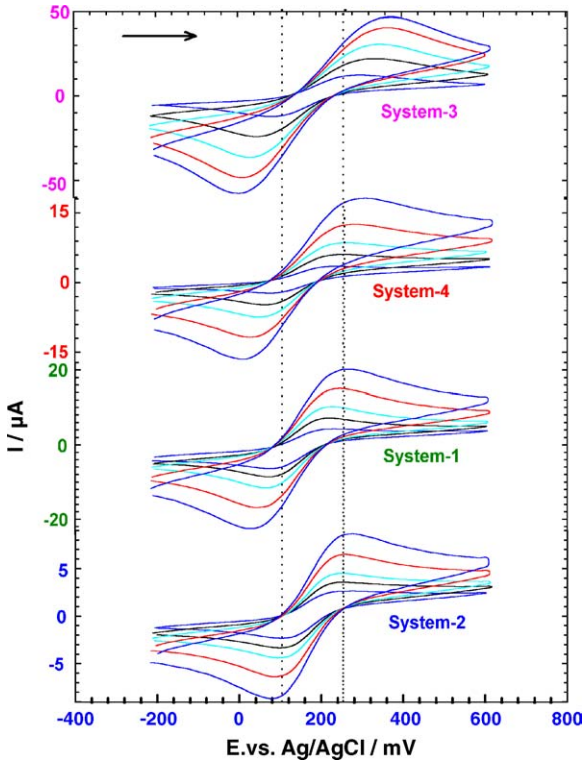

Fig. 1. Cyclic voltammograms of potassium ferricyanide encapsulated ormosil-modified electrodes prepared under four different conditions (Table 1) in 0.1 M phosphate buffer pH 7.0 at the scan rate of 5, 10, 20, 50 and 100 mV/s.

3.1. Electrochemistry of chemically sensitized ormosil-modified electrodes

Fig. 1 shows the electrochemistry of potassium ferricyanide encapsulated within these ormosil-modified electrodes (system-1 to system-4). Amongst all these modified electrodes, presence of Nafion within ormosil film caused better redox electrochemistry of ormosil encapsulated potassium ferricyanide. The peak separation is virtually independent of scan rate which is attributed to ion-exchange behavior of Nafion whereas the crown ether-modification showed relatively larger peak current and peak separation changes significantly on scan rate. Relatively greater peak separation in system-3 may be due to existence of ion-pair and charge separation is possibly introduced within film through the recognition of potassium ion by dibenzo-18-crown-6 whereas ferricyanide ions lie out side of the macrocyclic cavity. This generates an increase in capacitive current as supported by increase in capacitive components in system-3. It should be noted that duration of drying time caused dramatic effect on the electrochemical performances of the ormosil-encapsulated mediator. The drying time has been optimized under present experimental conditions together the contribution of laboratory's environment in order to get optimum electrochemical performances of ormosil-modified electrode. Low drying time under such conditions, i.e., 1–3 h

resulted, although smooth film over ITO electrode, however, the mediator got leached out from the film during electrochemical measurement. On the other hand, high drying time, i.e., >8–10 h resulted the film with poor electrochemical behavior of ormosil-encapsulated mediator. Such behavior of the modified electrode is expected due to the property of sol–gel reactions that ultimately approach to the state of xerogel with added property of densitification thereby causing restricted translational movement of the encapsulated mediator. We are working on these lines by introducing novel electro catalyst within nano-structured domain and detail out put would be communicated in later publication.

3.2. Chemistry of Prussian blue formation

Chemistry of electrode-4 is found very interesting which is prepared using the reaction product of THF/cyclohexanone, 3-aminopropyltrimethoxysilane and potassium ferricyanide. Potassium ferricyanide in the presence of 3-aminopropyltrimethoxysilane and either tetrahydrofuran

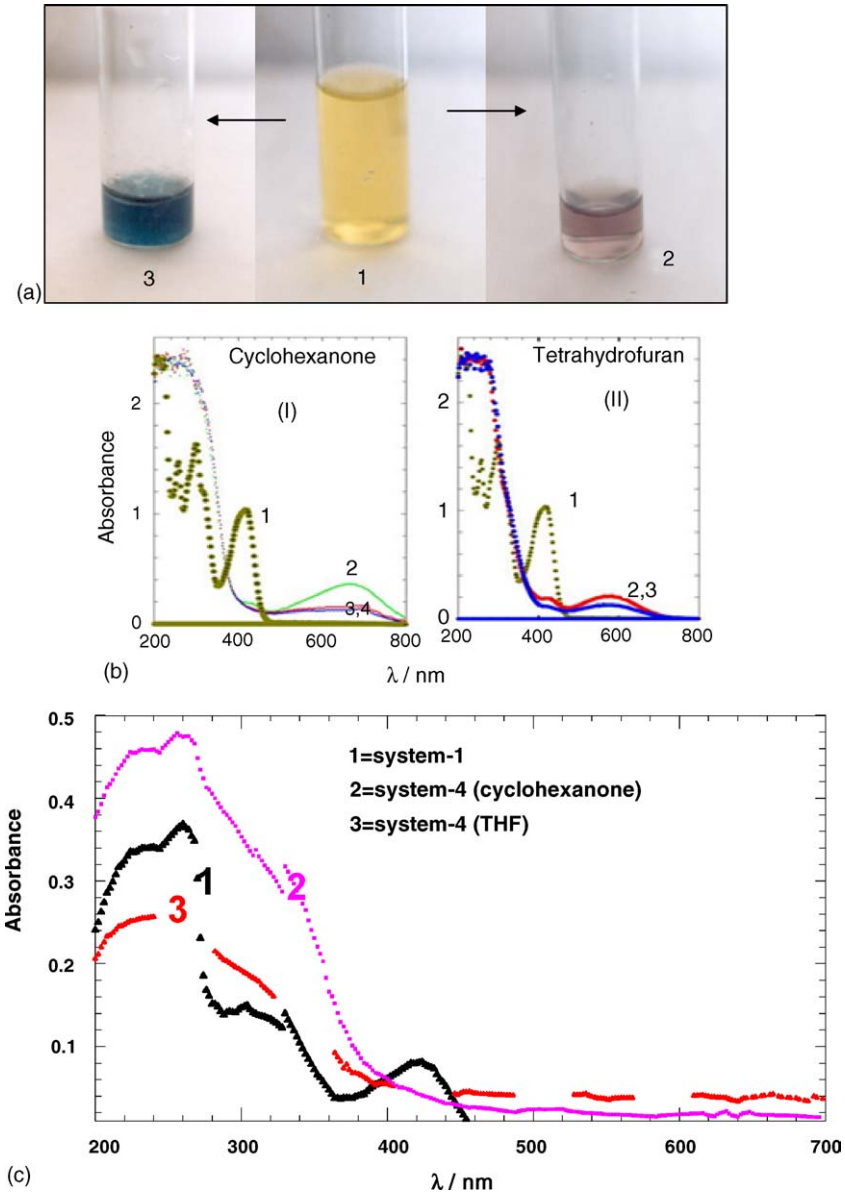

Fig. 2. (a) Colour change in the formation of charge-transfer complex: (1) potassium ferricyanide colour, (2) colour resulted with tetrahydrofuran and (3) colour resulted with cyclohexanone. (b) Absorption spectra of Prussian blue formation in the presence of cyclohexanone (I) and tetrahydrofuran (II). Curve-1 (I and II) shows the absorption spectra of the solution of potassium ferricyanide and 3-aminopropyltrimethoxysilane; curve-2 (I), the absorption after adding cyclohexanone [curve-3 and -4 (I) show recordings of same system at the interval of 5 min]; curve-2 (II), the absorption after adding tetrahydrofuran [curve-3 (II) shows recording of same system at the interval of 5 min]. (c) Absorption spectra of ormosil; curve-1 for system-1; curve-2 for system-4 (cyclohexanone) and curve-3 for system-4 (tetrahydrofuran). (d) Absorption spectra on the interaction of potassium ferricyanide and dibenzo-18-crown-6; curve-1 shows the absorption spectra of solution containing 10 mM potassium ferricyanide (30 μl), THF (5 μl), distilled water (965 μl) and curve-2 show the same with 10 mM potassium ferricyanide (30 μl), 1 mg/ml dibenzo-18-crown-5 in THF (5 μl), distilled water (965 μl). The inset show the spectra within 240–358 nm.

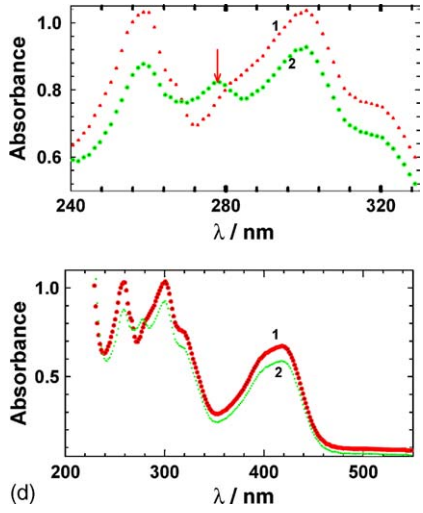

Fig. 2. (*Continued*).

or cyclohexanone under go Prussian blue formation and the yellow color of potassium ferricyanide changes depending on the nature of electron acceptor participating in Prussian blue formation as shown in Fig. 2a. Tetrahydrofuran generate light blue color whereas cyclohexanone result dark green colour. We have investigated the kinetics of such reaction spectrophotometrically and shown in Fig. 2b for cyclohexanone (I) and tetrahydrofuran (II). Fig. 2b (I) shows the variation of absorption maxima under three different conditions. Curve-1 (Fig. 2b (I) shows the absorption as a function of wavelength of the solution containing potassium ferricyanide and 3-aminopropyltrimethoxysilane before the addition of cyclohexanone whereas curve-2 to -4 [Fig. 2b (II)] show the absorption after the addition of cyclohexanone at the interval of 5 min in each case. Similarly, curve-1 (Fig. 2b (II) shows the absorption of the solution similar to that for curve-1 as reported in Fig. 2b (I) before the addition of tetrahydrofuran. The variation in the absorption spectra shown in Fig. 2b justify the following: (1) the absorption maxima recorded for potassium ferricyanide at 420 nm (curve-1 for both Fig. 2b (I) and (II) disappear; (2) the reaction of cyclohexanone to form Prussian blue is faster than tetrahydrofuran; (3) the reaction product with cyclohexanone causes reappearance of another peak around 675 nm [Fig. 2b (I) curve-2 and -3] whereas tetrahydrofuran generate new peak at 570 nm; (4) the absorption peak at both 570 and 675 nm get disappear kinetically; (5) variation in absorption in UV region is also seen in the reaction product of cyclohexanone and tetrahydrofuran. Such variation in the kinetics of cyclohexanone interaction reflects charge-transfer complexation during Prussian blue formation. Such conclusion is also supported by another spectrophotometric observation of ormosils without and with Prussian blue formation. Fig. 2c shows the absorption spectra of ormosil (control/system-1) (curve-1) and of ormosil [(system-4 cyclohexanone (curve-2) and tetrahydrofuran (curve-3). Disappearance of absorption at 420 nm (curve-1 as observed in system-1) and appearance of large plateau in UV region in system-4 in both cases (cyclohexanone and tetrahydrofuran) justifies the charge-transfer complexation in Prussian blue formation.

3.2.1. Interaction of potassium ferricyanide and dibenzo-18-crown-6

Another important aim to proceed ahead is to understand the interaction of potassium ferricyanide and dibenzo-18-crown-6. For this, we conducted few spectrophotomteric investigations as shown in Fig. 2d. Curve-1 (Fig. 2d) shows the spectra of solution containing potassium ferricyanide whereas curve-2 shows the spectra of the same solution with dibenzo-18-crown-6 recorded between 200 and 700 nm. The inset shows the variation of absorption between 240 and 328 nm. There are two major finding out of the observations: (1) there is appearance of a new peak closed to 280 nm; (2) the absorption at 420 nm which is characteristic of potassium ferricyanide that gets decreased after the addition of crown ether. These two findings justify the interaction of potassium ferricyanide and dibenzo-18-crown-6.

3.3. Sensing of dopamine at the surface of chemically sensitized electrodes

Fig. 3a shows the cyclic voltammograms of ormosil-modified electrodes (system-1, system-4) before and after the addition of 1 mM dopamine in 0.1 M phosphate buffer pH 7. The results show large difference in peak current in system-3 after the addition of 1 mM dopamine as compared to all other three systems. The order of sensitivity in term of peak current variation is found to be system-3 > system-4 > system-1 > system-2. The high sensitivity of system-3 is possibly due to facilitated diffusion of dopamine within ormosil as a result of ion pair existence in system-3 as compared to other three systems.

We again investigated the encapsulation of potassium ferrocyanide within the ormosil matrix and investigated the dopamine sensing. It should be noted that potassium ferrocyanide does not undergo Prussian blue formation in the presence of THF/cyclohexanone. The results on dopamine sensing using potassium ferrocyanide encapsulated ormosil are comparable to system-1, -2 and -3. The dopamine sensing is same order as observed with potassium ferricyanide modified electrode, however, the magnitude of current change after dopamine addition is less as compared to that of potassium ferricyanide under similar experimental condition. The question arises here what is nature of dopamine sensing on the electrode made without potassium ferricyanide/Prussian blue. Accordingly, we also investigated on these lines by preparing similar modified electrodes in absence of potassium ferricyanide Prussian blue and observed dopamine sensing under similar conditions. Fig. 3b shows the results on these four systems in absence of potassium ferricyanide. Here, it should be noted that the difference in system-3 and -4 was only in solubilizing the dibenzo-18-crown-6. In

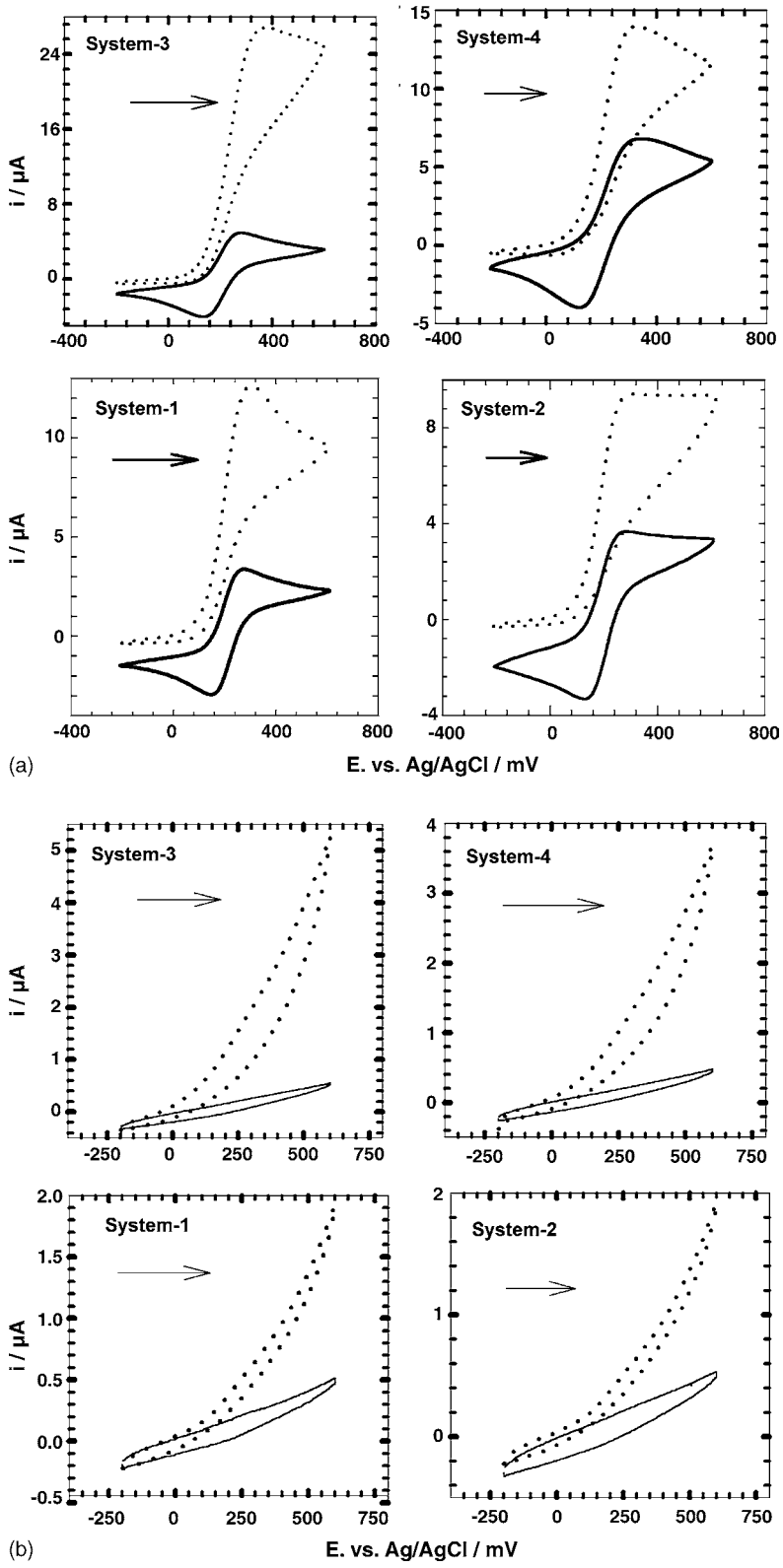

Fig. 3. (a) Cyclic voltammograms of system-1 to -4 before (1) and after the addition of 1 mM dopamine (2) in 0.1 M phosphate buffer pH 7.0 at the scan rate of 5 mV/s. (b) Cyclic voltammograms of system-1 to -4 made in absence of potassium ferricyanide. The system-3 and -4 have only difference of crown ether solubilization. The system-3 was made using crown ether dissolved in tetrahyrofuran whereas in system-4 crown ether was dissolved in phenyltrimethoxysilane. Prussian blue was absent in system-4. Results show before (1) and after the addition of 1 mM dopamine (2) in 0.1 M phosphate buffer pH 7.0 at the scan rate of 5 mV/s.

system-3, this was dissolved in tetrahydrofuran whereas in system-4, it was present in phenyltrimethoxysilane. It could be concluded from the results (Fig. 3b) that the presence of doibenzo-18-crown-6 causes amplification in dopamine sensing which is in accordance to the observations made using potassium ferricyanide/Prussian blue encapsulation.

3.4. Effect of Dibenzo-18-crown-6 concentration on dopamine sensing

The results reported in Fig. 3 suggested that the presence of dibenzo-18-crown-6 caused amplification in dopamine sensing as compared to that recorded for system without the same. Further, the results reported in Fig. 3 also revealed that system-4 even in absence of dibenzo-18-crown-6 also responded nicely with relatively much better amplification as compared to that of system-1 and -2. Accordingly, we monitored dopamine sensing on system-5 made with the presence of Prussian blue and varying concentrations of dibenzo-18-crown-6 (system-5a, -5b, -5c and -5d) (Fig. 4). It should be noted that the preparation of ormosil films is triggered by conversion of potassium ferricyanide into Prussian blue at first instant followed by addition of dibenzo-18-crown-6 solution in tetrahydrofuran of desired concentrations and other necessary additives as shown in Table 1C. Fig. 4 shows the results on dopamine sensing on system-5a, -5b, -5c and -5d. The results recorded in Fig. 4 justify the significance of the crown ether on dopamine detection. Some important findings are as follows: (1) in the presence of crown ether the amplification appreciable; (2) the response on dopamine sensing increases on increasing crown ether concentrations upto 1 mg/ml and further rise in crown ether concentration in the film resulted decrease in response; (3) maximum response is recorded with the ormosil film made with 1 mg/ml dibenzo-18-crown-6; (4) absence of crown ether shows poor dopamine sensing (system-1) followed by strong interference from ascorbic acid. These finding again conformed our conclusion on the contribution of dibenzo-18-crown-6 on dopamine sensing. We used the system as referred system-5b for the analysis of dopamine.

Fig. 5 shows typical results on the additions of varying concentrations of dopamine using system-5c made using 1 mg/ml dibenzo-18-crown-6 together ormosil precursors. Excellent dopamine sensing is recorded with much faster response time to the order of <10 s. Inset to Fig. 5 shows the calibration curve for the same. The lowest detection limit is recorded to be 30 nM which is much superior as compared to any electrochemical systems available right now in literature on similar approach. Such large amplification in dopamine sensing could be explained from following consideration. Dibenzo-18-crown-6 is an excellent carrier of potassium ion. Accordingly, the potassium ion of redox species

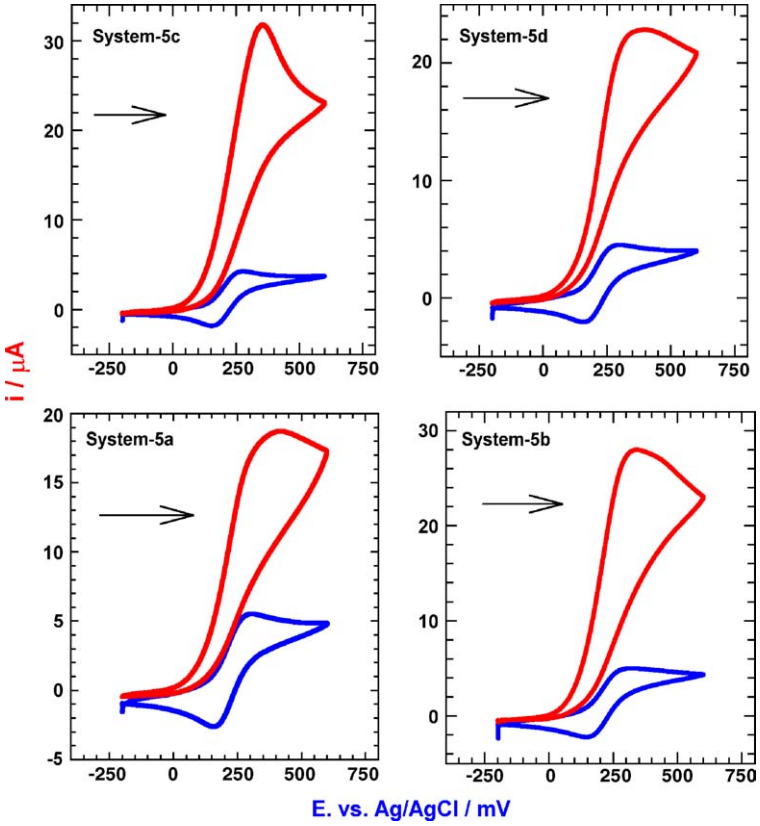

Fig. 4. Cyclic voltammograms of system-5a, -5b, -5c and -5d before (1) and after the addition of 0.5 mM dopamine (2) in 0.1 M phosphate buffer pH 7.0 at the scan rate of 5 mV/s.

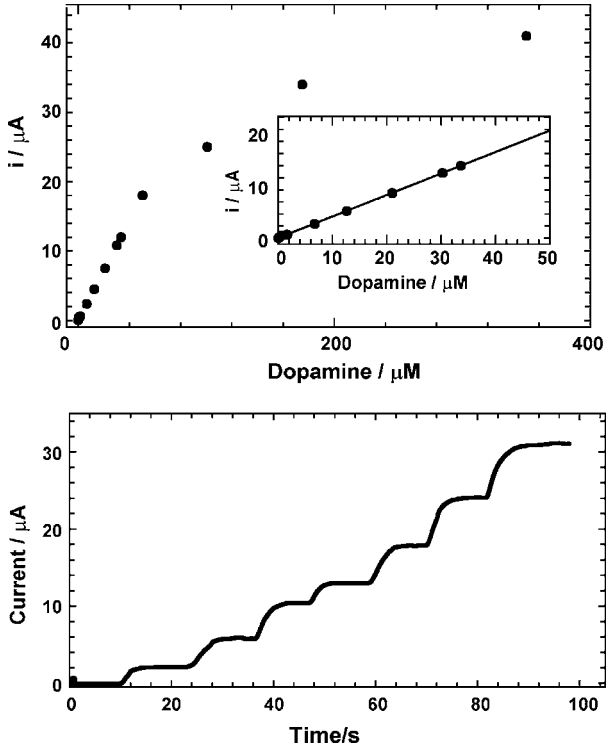

Fig. 5. Typical response curve of sytem-3 on the addition of increasing concentrations of dopamine in 0.1 M phosphate buffer pH 7.0. The electrode was polarized at 200 mV vs. Ag/AgCl. The inset shows the calibration curve for dopamine analysis using system-5c.

$[K_3Fe(CN)_6]$ lies within the cavity and ferricyanide anions are distributed within rest of the film. The presence of anion within film facilitates the diffusion of dopamine cation within the solid-state ormosil film and accordingly crown-ether dependent amplification in dopamine sensing is recorded.

3.5. Selectivity in dopamine sensing

The analysis of dopamine is normally corrupted due to the presence of other electro active species especially ascorbic acid present in physiological fluid. We again investigated the dopamine sensing in the presence of ascorbic acid and is recorded in Fig. 6. Curve-1 shows the results before the addition of ascorbic acid whereas curve-2 shows the same after the addition of 1 mM ascorbic acid which is around three-fold greater than the normal physiological ascorbic acid concentration. After the addition of ascorbic acid, instead of increase in amperometric response the anodic current get decreased (curve-2; Fig. 6). When dopamine (0.2 mM) is added in the system after ascorbic acid addition again there is enough response on dopamine sensing which justifies that the system is not corrupted by ascorbic acid. However, when the ormosil-modified electrode without dibenzo-18-crown-6 was used to monitor dopamine, the electrode responded to ascorbic acid. Accordingly, the selectivity of dopamine sensing was justified from the participation of dibeno-18-crown-6. Insensitivity of ascorbic acid to the present system could be explained

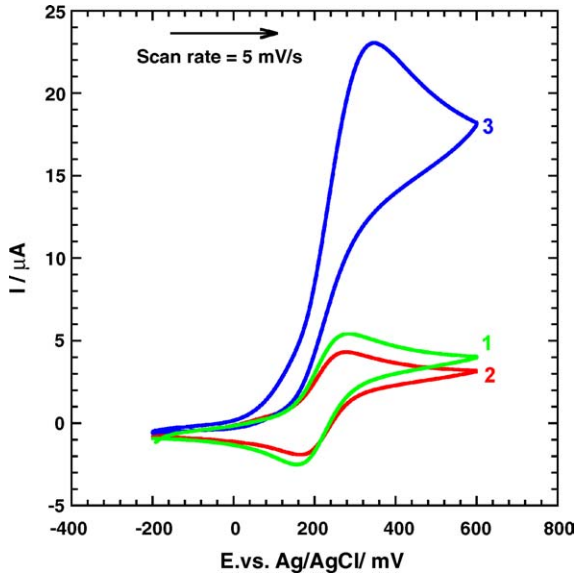

Fig. 6. Cyclic voltammograms of system-5 before (curve-1) and after the addition of 0.2 mM ascorbic acid (curve-2) followed by addition of 0.4 mM dopamine in 0.1 M phosphate buffer pH 7.0 at the scan rate of 5 mV/s.

as follows. The presence of anion within the ormosil matrix restricts the diffusion of ascorbate anion within the ormosil. Accordingly, reverse in the diffusion of ascorbic acid kinetics as compared to dopamine diffusion kinetics prevail, which justify the importance of present system for dopamine sensing. We also observed that increase in crown ether concentration caused relative decrease in ascorbic acid sensing that again justify the contribution of dibenzo-18-crown-6 in dopamine sensing. System-2 also not responded to ascorbic acid which is in accordance to the property of Nafion restricting diffusion of ascorbic acid within the film. However, system-1 and -4 responded to ascorbic acid significantly. The studies on the interferences from other species and the practical applications of the sensor are underway and will be reported in later publication. It is further necessary to talk on the miniaturization of the sensor into a portable device. The recent growth on optrode's design will possibly be helpful in this direction as opto-electrochemistry has shown powerful tool during recent year and the idea behind which could be emanated from the power of neural network in living system.

Since the present ormosil-modified electrodes do not incorporate any biological molecules the stability of the electrodes are quite excellent, however, the storage condition of the electrode is very crucial. The electrodes are quite stable for six months retaining close to 99% reproducibility even after six months when stored in working buffer.

4. Conclusion

We report herein few chemically sensitized electrodes for electro analytical applications. Five system that differ in

each other in the presence of potassium ferricyanide (system-1), potassium ferricyanide and Nafion (system-2), potassium ferricyanide and dibenzo-18-crown-6 (system-3), Prussian blue only (system-4) and Prussian blue with dibenzo-18-crown-6 (system-5). These modified electrodes were used for dopamine sensing. It was found that the presence of dibenzo-18-crown-6 caused better amplification in dopamine sensing. The conversion of Prussian blue within ormosil (system-4) resulted enhanced dopamine sensing as compared to that of potassium ferricyanide only (system-1) and also system-2 which was further enhanced due to the presence dibenzo-18-crown-6 that caused facilitated diffusion of dopamine within the ormosil film. The presence of crown ether also caused selectivity in dopamine sensing especially in the presence of ascorbic acid mainly based on the restricted diffusion of ascorbate ion within the ormosil film.

Acknowledgments

The authors are thankful to DST New Delhi (grant number SR/S1/PC-31/2003) for financial support. B.C.U. is thankful to CSIR or the award of Research Associateship.

References

[1] M.M. Collinson, A.R. Howells, Anal. Chem. 72 (2000) 702A–709A.
[2] P.C. Pandey, S. Upadhyay, H.C. Pathak, Electroanalysis 11 (1999) 59–65.
[3] P.C. Pandey, S. Upadhyay, I. Tiwari, V.S. Tripathi, Sens. Actuators B 75 (2001) 48–55.
[4] P.C. Pandey, S. Upadhyay, N.K. Shukla, S. Sharma, Biosens. Bioelectron. 18 (2003) 1257.
[5] P.C. Pandey, S. Upadhyay, S. Sharma, J. Electrochem. Soc. 150 (2003) H85–H92.
[6] L. Zhang, N. Teshima, T. Hasebe, M. Kurihara, T. Kawashima, Talanta 50 (1999) 677–683.
[7] B.K. George, in: L.S. Goodman, A. Gilman (Eds.), The Pharmacological Basis of Therapeutics, third ed., The Macmillan Co., New York, 1965, p. 427.
[8] G. Aymard, B. Labarthe, D. Warot, I. Berlin, B. Diquet, J. Chromatogr. B 744 (2000) 25–31.
[9] Y. Wang, D.S. Fice, P.K.F. Yeung, J. Pharm. Biomed. Anal. 21 (1999) 519–525.
[10] R.P.H. Nikolajsen, A.M. Hansen, Anal. Chim. Acta 449 (2001) 1–15.
[11] D.P. Nikolelis, M. Mitrokotsa, Biosens. Bioelectron. 17 (2002) 565–572.
[12] D.P. Nikolelis, S.-S. Petropoulou, Electroanalysis 14 (2002) 783–790.
[13] D.P. Nikolelis, S.-S. Petropoulou, Biochim. Biophys. Acta 1558 (2002) 238–245.
[14] D.P. Nikolelis, S.-S. Petropoulou, G. Theoharis, Electrochim. Acta 47 (2002) 3457–3467.
[15] J. Wang, J. Liu, Anal. Chim. Acta 294 (1994) 201–206.
[16] K. Odashima, K. Yagi, K. Yohda, Y. Umezawa, Bioorg. Med. Chem. Lett. 9 (1999) 2375–2378.
[17] D.P. Nikolelis, D.A. Drivelos, M.G. Simantiraki, S. Koinis, Anal. Chem. 76 (2004) 2174–3180.
[18] M. Ferreira, L.R. Dinelli, K. Wohnrath, A.A. Batista, O.N. Oliveira Jr., Thin Solid Films 446 (2004) 301–306.
[19] K. Hayashi, Y. Iwasaki, R. Kurita, K. Sunagawa, O. Niwa, Electrochem. Commun. 5 (2003) 1037–1042.
[20] R. Kataky, E. Morgan, Biosens. Bioelectron. 18 (2003) 1407–1417.
[21] M.D. Pilar, T. Sotomayor, A.A. Tanaka, L.T. Kubota, Electrochim. Acta 48 (2003) 855–865.
[22] D.R. Shankaran, K. Iimura, T. Kato, Sens. Actuators B: Chem. 94 (2003) 73–80.
[23] D.R. Shankaran, N. Uehara, T. Kato, Anal. Chim. Acta 478 (2003) 321–327.
[24] M.D. Pilar, T. Sotomayor, A.A. Tanaka, L.T. Kubota, Anal. Chim. Acta 455 (2002) 215–223.
[25] L. Mao, K. Yamamoto, Anal. Chim. Acta 415 (2000) 143–150.
[26] F. Lisdat, U. Wollenberger, A. Makower, H. Hörtnagl, D. Pfeiffer, F.W. Scheller, Biosens. Bioelectron. 12 (1997) 1199–1211.
[27] Y. Chen, T.C. Tan, Chem. Eng. Sci. 51 (1996) 1027–1042.
[28] Y. Chen, T.C. Tan, Sens. Actuators B: Chem. 28 (1995) 39–48.
[29] Y. Chen, T.C. Tan, Biosens. Bioelectron. 9 (1994) 401–410.
[30] M.A. Dayton, A.G. Ewing, R.M. Wightman, J. Electroanal. Chem. 146 (1983) 189–200.
[31] A.G. Ewing, R.M. Wightman, M.A. Dayton, Brain Res. 249 (1982) 361–370.
[32] M.D. Pilar, T. Sotomayor, A.A. Tanaka, L.T. Kubota, J. Electroanal. Chem. 536 (2002) 71–81.
[33] Y. Chen, T.C. Tan, Talanta 42 (1995) 1181–1188.
[34] J. Wang, Talanta 41 (1994) 857–863.
[35] J. Wang, L. Chen, Biosens. Bioelectron. 11 (1996) 751–756.
[36] P.C. Pandey, B.C. Upadhyay, A.K. Upadhyay, Anal. Chimica. Acta 523 (2) (2004) 219–223.