Nano-composition of riboflavin–nafion functional film and its application in biosensing

S Rezaei-Zarchi[1,2], A A Saboury[1,*], A Javed[3], A Barzegar[1], S Ahmadian[1] and A Bayandori-Moghaddam[4]

[1]*Institute of Biochemistry and Biophysics, University of Tehran, Tehran, Iran*
[2]*Department of Biology, Payam-e-Noor University, Yazd, Iran*
[3]*Department of Biochemistry, University of Agriculture, Faisalabad, Pakistan*
[4]*Department of Chemistry, Faculty of Sciences, University of Tehran, Tehran, Iran*

**Corresponding author (Fax, +98-21-66404680; Email, saboury@ut.ac.ir)*

A novel nafion–riboflavin membrane was constructed and characterized by the scanning electron microscopy (SEM), transmission electron microscopy (TEM), UV-visible spectroscopy and cyclic voltammetric techniques. The estimated average diameter of the designed nanoparticles was about 60 nm. The functional membrane showed a quasi-reversible electrochemical behaviour with a formal potential of –562 ± 5 mV (vs Ag/AgCl) on the gold electrode. Some electrochemical parameters were estimated, indicating that the system has good and stable electron transfer properties. Moreover, horseradish peroxidase (HRP) was immobilized on the riboflavin–nafion functional membrane. The electrochemical behaviour of HRP was quasi-reversible with a formal potential of 80 ± 5 mV (vs Ag/AgCl). The HRP in the film exhibited good catalytic activity towards the reduction of H_2O_2. It shows a linear dependence of its cathodic peak current on the concentration of H_2O_2, ranging from 10 to 300 μM.

[Rezaei-Zarchi S, Saboury A A, Javed A, Barzegar A, Ahmadian S and Bayandori-Moghaddam A 2008 Nano-composition of riboflavin–nafion functional film and its application in biosensing; *J. Biosci.* **33** 279–287]

1. Introduction

It is well known that biological macromolecules exhibit a rather slow rate of heterogeneous electron transfer at conventional electrodes, ascribed to either their extended three-dimensional structures or the resulting inaccessibility of the electro-active centre or to their adsorption onto and subsequent passivity of the electrode surface (Scheller *et al* 2005). In recent years, numerous investigations have been carried out on electrochemical biosensors to improve the rapidity, selectivity and sensitivity, and reduce the cost of chemical analysis (Khoo and Chen 2002; Kurzawa *et al* 2002; Kong *et al* 2003). Much effort has been focused on facilitating electron transfer between proteins and electrodes, including the application of electron transfer mediators, where immobilization of the mediator is as important as that of the enzyme (Chaubey and Malhotra 2002). A series of organic materials such as methylene blue (Arvand *et al* 2003), methylene green (Munteanu *et al* 2001), Prussian blue (Karyakin *et al* 2004), phenazines (Pessoa *et al* 1997) and thionine (John and Ramaraj 2004) have been used as electron transfer mediators. Flavins work as redox coenzymes in many biological transformations and hence they have been used as redox mediators between the electrode and several enzymes (Ogino *et al* 1995).

Materials that allow the immobilization of mediators and biological molecules have a great potential for the development of sensors and biosensors (Gorton *et al* 1991). Several studies using zeolites, clays, zirconium phosphate and modified silica as immobilization supports have been

Keywords. Biosensor; functional membrane; horseradish peroxidase; nanoparticles

Abbreviations used: HRP, horseradish peroxidase; nafion, nafion perfluorosulphonated ion-exchange resin; SEM, scanning electron microscopy; TEM, transmission electron microscopy

carried out successfully and published (Rolison 1990; Kubota *et al* 1995; Cai *et al* 1996). During recent years, the increasing popularity of nafion perfluorosulphonated ion-exchange resin (nafion) for the fabrication of redox polymer-modified electrodes is due to the easy fabrication, good electrical conductivity, and high partition coefficients of many redox compounds in nafion, which is a perfluorinated anionic polyelectrolyte. At present, reagent grafting onto a nafion surface is a common procedure in order to obtain supports for the catalysts, ion-exchange selectivity from the solution and matrices for immobilization of several materials, such as enzymes and electron mediators (Miura *et al* 1984; Gogol *et al* 2000). These properties make nafion an excellent matrix for use in modified electrodes. Nafion has some important characteristics such as a high surface area, rigidity, porosity, chemical stability, good biocompatibility and the ability to resist interference from anions and biological macromolecules, making it a promising matrix (Murray 1984; Andrieux *et al* 1990; Liu and Deng 1995).

The nafion immobilization process is simple and versatile, and offers good retention of the biochemical and recognition properties, increases the stability and also improves the electrochemical properties of the immobilized species. Considering these aspects, the present work describes the use of nafion for riboflavin immobilization, which is a new method for immobilizing riboflavin onto the surface of a gold electrode with the help of nafion. The combined stabilities of riboflavin and nafion, and the effect of electrolytes are also investigated in this study. In addition, horseradish peroxidase (HRP) was immobilized onto the riboflavin–nafion film and applied to determine the concentration of H_2O_2.

2. Material and methods

2.1 *Reagents*

Riboflavin, nafion (5% ethanol solution), HRP and fine alumina powder were purchased from Sigma, St Louis, USA. Potassium dihydrogen phosphate, di-potassium hydrogen phosphate.$3H_2O$, HCl and $NaHCO_3$ were obtained from Merck, Darmstadt, Germany. All the reagents were of analytical grade. Solutions were prepared in deionized double distilled water (18 MΩ cm, Barnstead Instrument).

2.2 *Apparatus and measurements*

Electrochemical measurements were carried out with a potentiostat/galvanostat (Model 263A, EG&G, USA) by the cyclic voltammetric method (cyclic voltammetry is a type of potentio-dynamic electrochemical measurement in which the voltage is applied to a working electrode in the solution and the current, flowing towards the working electrode, is plotted versus the applied voltage to give the cyclic voltammogram) and a single-compartment voltammetric cell equipped with a platinum rod auxiliary electrode, an Ag/AgCl reference electrode (from Metrohm) and a working gold electrode, with a disk diameter of 1 mm (Azar Electrode, Iran). Scanning electron microscopy (SEM) and transmission electron microscopy (TEM) images were obtained with DSM 960A and CEM 902A (Zeiss, Germany), respectively.

2.3 *Nafion–riboflavin-modified gold electrode*

For the preparation of the nafion–riboflavin-modified gold electrode, the concerned electrode was mechanically polished twice with alumina (particle sizes 10 and 0.3 μm) to a mirror finish. Then, it was ultrasonically treated in water for 10 min. Thereafter, it was treated electrochemically in 0.2 M sulphuric acid, cycling between –0.2 and +1.5 V (vs Ag/AgCl) at a sweep rate of 0.1 Vs^{-1}, until a clean gold electrode was obtained. Finally, the electrode was washed with deionized double distilled water. Nafion solution (2 μl, 5%) was dropped onto the surface of the freshly prepared gold electrode and dried at room temperature for 20 min. Then, the electrode was dipped into a freshly prepared riboflavin solution (1 mM) for 10 min, washed carefully with deionized double distilled water and stored at 4°C when not in use. The experimental solutions were de-aerated using highly pure nitrogen for 30 min and a nitrogen atmosphere was kept over the solutions during the whole measurement. All the electrochemical experiments were carried out in 0.1 M phosphate buffer solution, pH 7.0 at 25 ± 1°C.

2.4 *Electron microscopy of the membrane*

In order to obtain the SEM images, the samples were prepared by the method described for the preparation of a nafion–riboflavin-modified gold electrode. The TEM images of the nafion–riboflavin particles were taken by the following method: at first, a solution with the same volumes of 1 mM riboflavin and 5% nafion was prepared and diluted 100 times with 50% ethanol. A drop of this diluted solution was added to fomvar/carbon-coated grids (400 meshes) and after drying, viewed under the TEM, operating at 80 kV.

2.5 *HRP–nafion–riboflavin modified gold electrode*

Of the HRP solution (10 mg/ml in pure water) 2 μl was dropped onto the nafion–riboflavin-modified gold electrode and dried in air at room temperature for about 60 min. The modified electrode was kept in a bottle over water vapour at 4ºC when not in use.

3. Results and discussion

3.1 *Material characterization*

The riboflavin–nafion membrane surface was viewed with SEM and TEM to consider its uniformity and other characteristics. The SEM images, taken from a cross-section of the membrane, represent the interaction between the substrate and the riboflavin–nafion membrane. Figure 1a demonstrates that the membrane is in close contact with the substrate and has a thickness of about 800–1200 nm. The top view of the membrane showed several meshy protuberances and hollow structures (figure 1b). The TEM images showed that the nafion–riboflavin particles were in a mixture of integrated and single-particle forms, made from uniform spheres with an average particle size of about

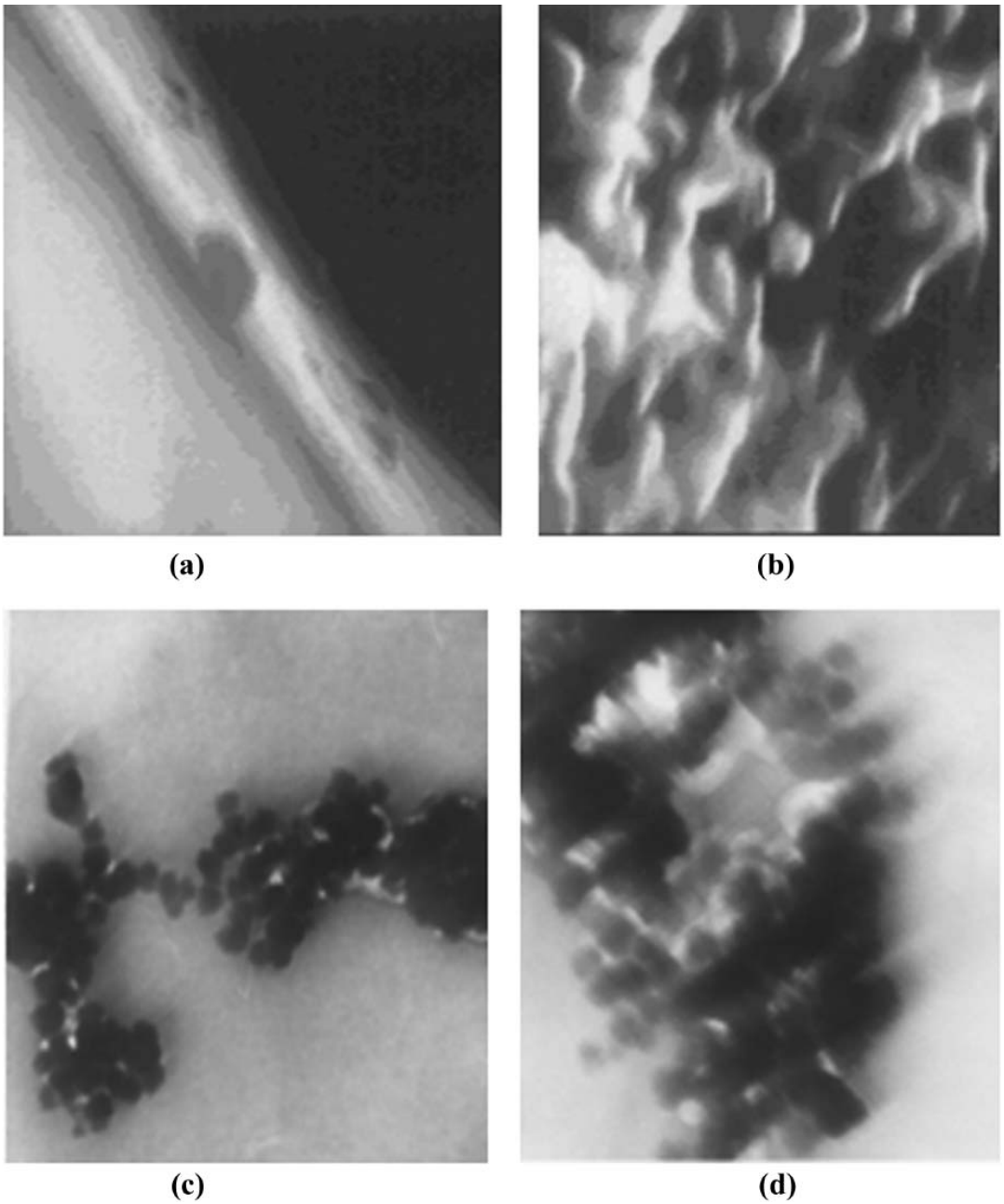

Figure 1. Scanning and transmission electron micrographs (TEM) of nafion and riboflavin. Scanning electron micrograph images of the nafion–riboflavin membrane: **(a)** cross-section, **(b)** top view. TEM images **(c, d)** of nafion–riboflavin nanoparticles. (For details, refer to the text.)

60 nm (figure 1c,d). In fact, riboflavin has the potential to be immobilized strongly onto the electrode surface and the riboflavin–nafion-modified electrode is not only stable but according to Chen *et al* (1994), these nanoparticle structures also greatly increase the surface area of the membrane. On the other hand, riboflavin molecules disperse homogeneously inside the nanoparticles (Yao *et al* 2005).

Figure 2 shows the UV-visible spectra of riboflavin, adsorbed on nafion (a) and in the solution (b), respectively. By adding riboflavin to nafion, no changes were observed on the status of riboflavin peaks at 364 and 440 nm but the adsorption was decreased to considerable levels. As described before, micro-emulsion and nanoparticle production of the materials decreases their absorbance in the UV-visible region (Clarke *et al* 1997; Yao *et al* 2005; Gao *et al* 2006). Hence, the results show that nafion particles gather around some of the riboflavin molecules and develop nanoparticles. The structure of nafion has an abundance of F and O atoms, which can develop very strong hydrogen bonds with some H atoms in the riboflavin molecules. In the present study, electron microscopy showed the construction of nanoparticles while the UV-visible spectra verified these findings. Our previous study, based on the production of methylene blue nanoparticles on nafion film (Hong *et al* 2007), and the recent investigation has proven that nafion can be a very interesting and useful material in the construction of nanoparticles used in different electroactive materials.

3.2 *Electrochemical properties of the material*

Cyclic voltammograms obtained for the nafion-modified gold electrode did not show any peak current in the investigated potential range, while in case of the nafion–riboflavin-modified gold electrode, a clear redox peak was scanned that indicates immobilization of riboflavin on nafion. This observation represents a good electrochemical signal for the present analysis. The formal potential ($E^{o\prime}$), determined by using the following equation: $E^{o\prime} = (E_{pc} + E_{pa})/2$ (where E_{pc} is the cathodic and E_{pa} is the anodic peak potential), was about –562 mV at pH 7.0. A linear dependence of the anodic and cathodic peak currents on the square root of the scan rates ($v^{1/2}$) is illustrated in figure 3 for the adsorbed riboflavin. This behaviour is similar to a diffusion-controlled redox process but no decrease in the peak current was observed after repeated cycles of this experiment (figure 4). These findings indicate that riboflavin is strongly adsorbed onto the surface of nafion. Hence, the redox process is controlled by the diffusion of counter-ions to maintain electroneutrality on the electrode surface. Other possible causes for this behaviour can be the resistance of the material or an electron transfer process, occurring by the electrohopping mechanism (Yamashita *et al* 2002). This behaviour is more likely to be seen when the electron transfer mechanism occurs at a high concentration of the supporting electrolyte.

The separation of the peak potentials, ΔE_p ($\Delta E_p = E_{pa} - E_{pc}$), was about 95 mV for riboflavin–nafion. It was almost constant with changes in scan rate. In addition, the ratios between the cathodic and anodic peak currents were near unity. This behaviour shows a good electron transfer rate between the adsorbed riboflavin and the electrode surface (Laviron 1982; Honeychurch and Rechnitz 1998). The number of electrons transferred between the electrode and the adsorbed riboflavin during the redox process was calculated by the use of the following equation: $E_{fwhm} = 90.6/n$, where n is the number of electrons participating in the reaction and E is the peak width at half height. For riboflavin adsorbed in this condition, the number of electrons was close to 1.5, which indicates that a part of the flavins is being converted to a semi-quinone state during the redox process. The present data are in accordance with a previous study done on riboflavin immobilization on a zirconium oxide-modified silica gel (Yamashita *et al* 2002).

Table 1 shows the effect of different ions on the nafion–riboflavin film. These ions can affect the electrochemical response of the electrode due to interaction with the adsorbed electro-active species or with the matrix (Yamashita *et al* 2002). Table 1 explains the effect of different electrolytes on $E^{o\prime}$ and ΔE_p, and also the results obtained from the average of the five measurements. Our data explain the fact that different ions do not change $E^{o\prime}$ and ΔE_p to a considerable level so they cannot be adsorbed by this film.

3.3 *Electrochemical characteristics of the HRP–nafion–riboflavin modified gold electrode*

Figure 5a represents the electron transfer properties of the immobilized HRP on the nafion–riboflavin-modified gold electrode at different sweep rates. A pair of well-defined redox peaks was observed in the potential range of

Table 1. Effect of different supporting electrolytes on $E^{o\prime}$ and ΔE_p for nafion–riboflavin obtained in 0.1 M solution at pH 7.0; scan rate 100 mV/s

Supporting electrolytes	ΔE_p	$E^{o\prime}$
KNO_3	87 ± 6	–555 ± 10
$LiNO_3$	95 ± 5	–560 ± 8
$NaNO_3$	94 ± 7	–548 ± 11
$Ca(NO_3)_2$	86 ± 7	–552 ± 10
NH_4NO_3	84 ± 9	–549 ± 6
NaCl	91 ± 7	–552 ± 7
Na_2SO_4	89 ± 9	–557 ± 11
$NaClO_4$	85 ± 8	–552 ± 9

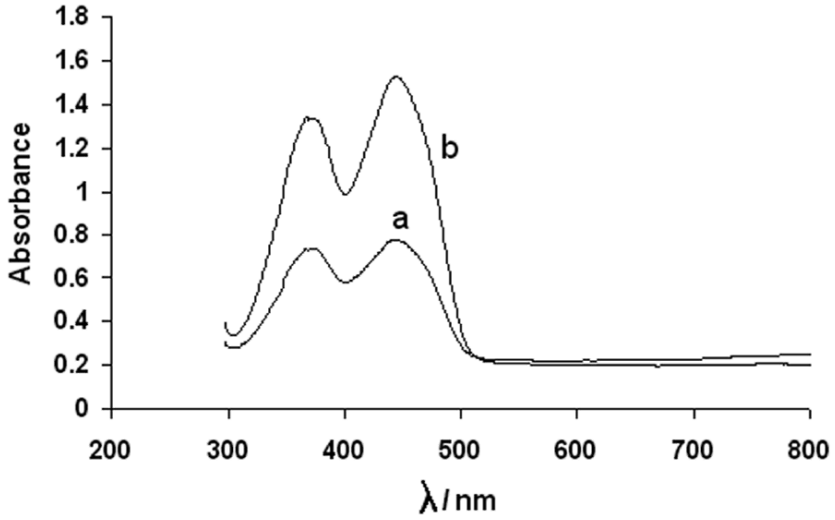

Figure 2. UV-visible spectra of riboflavin adsorbed on nafion **(a)** and in solution **(b)**.

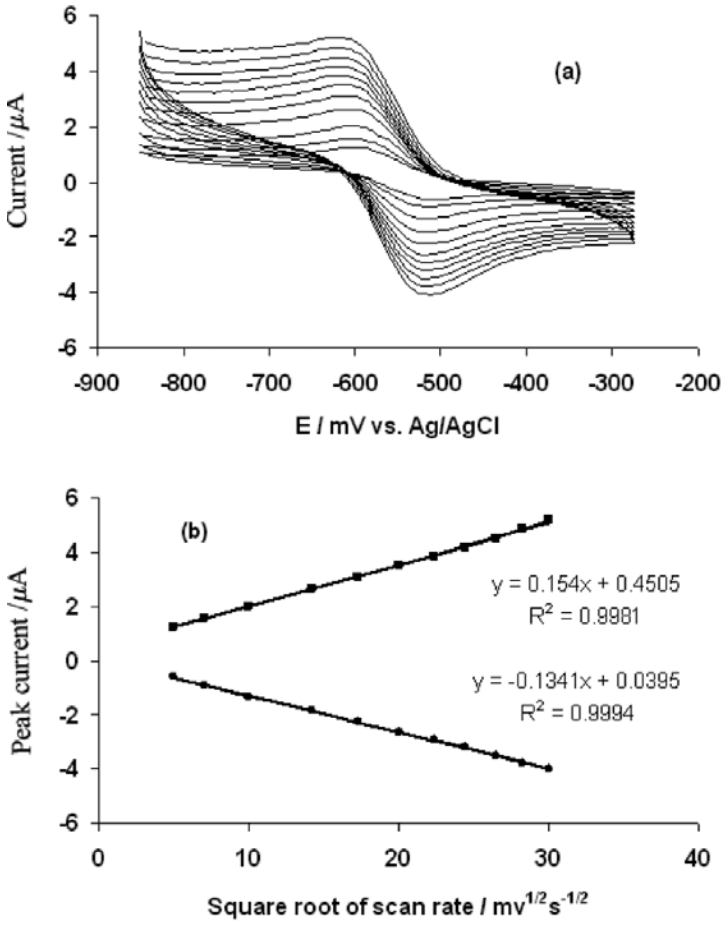

Figure 3. (**a**) Typical cyclic voltammograms of nafion–riboflavin on the gold electrode at different scan rates. The voltammograms (from inner to outer) designate scan rates of 25, 50, 100, 200, 300, 400, 500, 600, 700, 800, 900 and 1000 mV/s, respectively. (**b**) Dependence of the anodic and cathodic peak currents on the square root of the scan rates. All the data were obtained at pH 7.0 and 0.1 mol l^{-1} phosphate buffer solution.

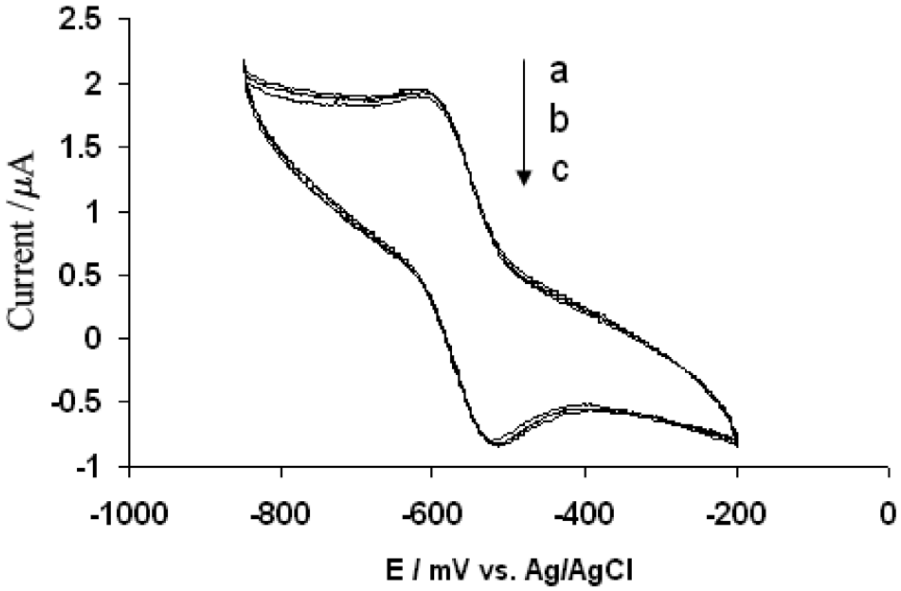

Figure 4. Cyclic voltammograms of riboflavin–nafion after **(a)** 1 cycle, **(b)** 50 cycles and **(c)** 100 cycles in 0.1 mol l^{-1} phosphate buffer solution at a scan rate of 100 mV s^{-1}.

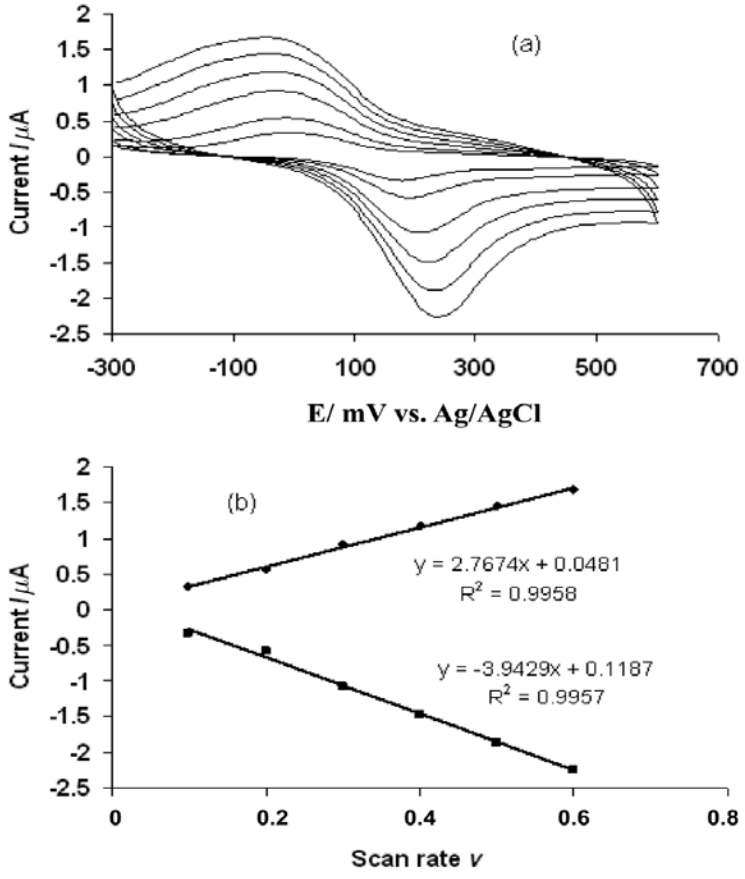

Figure 5. **(a)** Cyclic voltammograms of the nafion–riboflavin–horseradish peroxidase (HRP) modified gold electrode in 0.1 mol l^{-1} phosphate buffer solution at different sweep rates (from inner to outer: 0.1, 0.2, 0.3, 0.4, 0.5 and 0.6 V s^{-1}). **(b)** Relationship of peak current and scan rate (*v*).

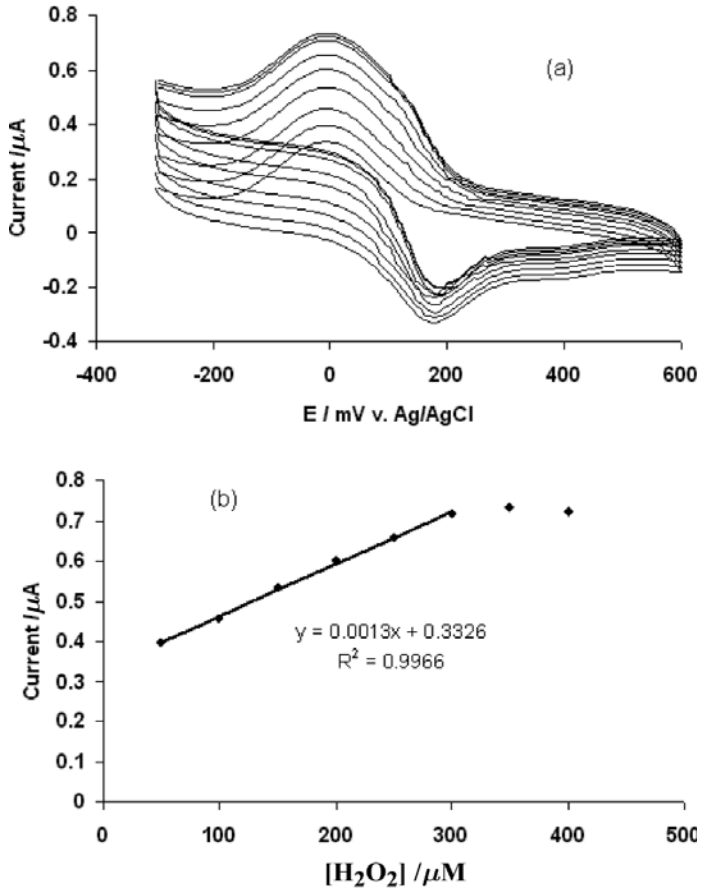

Figure 6. **(a)** Cyclic voltammograms obtained at a nafion–riboflavin– horseradish peroxidase (HRP) modified gold electrode in 0.1 mol l^{-1} phosphate buffer solution (pH 7.0) for 50–400 μM concentrations of H_2O_2 and **(b)** the relationship between the cathodic peak current of HRP and different concentrations of H_2O_2.

–0.3 to +0.6 V, which could be ascribed to the electron transfer of HRP at the modified electrode at different sweep rates. Figure 5b shows that the cathodic and anodic peak currents of HRP have a linear relationship with the scan rate. The integration of the redox peaks at different sweep rates also gave a nearly constant charge (Q) value. These are the characteristics of diffusionless and thin-layer electrochemical behaviour (Murray 1984), which showed good and stable immobilization of HRP onto the nafion–riboflavin film. Electron transfer of redox protein at the bare gold electrode is very slow so the redox peak cannot usually be observed (McNeil *et al* 1992; Tian *et al* 2004). Furthermore, no redox peaks were observed for the redox proteins at the nafion-modified gold electrode (without riboflavin) nor for the nafion–riboflavin modified gold electrode at a potential range of –0.3 to +0.6 V. However, the nafion–riboflavin functional membrane significantly facilitates the electron-transfer rates of the protein. Nafion improves the stability of HRP and offers a biocompatible microenvironment on the electrode surface (Chen *et al* 1994; Hong *et al* 2007).

Figure 6a represents the incorporation of HRP onto the nafion–riboflavin membrane, which functions as an effective catalyst for the reduction of H_2O_2. The calibration curve (figure 6b) shows a linear dependence of the cathodic peak current on the H_2O_2 concentration at a potential range of 0–300 μM. The reproducibility of the catalytic current response of the disposable sensor was tested at an H_2O_2 concentration of 50 μM. The sensor showed an acceptable reproducibility with a relative standard deviation of 2.6% for over 4 successive assays and the detection limit was 10 μM. The detection limit was determined from the cross point of the lines fitted to the linear segments of the cathodic peak current vs H_2O_2 concentration (Richard and Erno 1994). Thus, this experiment has introduced a new biosensor for the sensitive determination of H_2O_2 in solution.

4. Conclusions

The results show that nafion was able to immobilize riboflavin onto the surface of a gold electrode with good

stability and effective electron transfer. TEM and UV-visible spectroscopic analyses showed the incorporation of riboflavin nanoparticles onto the nafion polymer. We found that such incorporation facilitates electron transfer between the protein and the electrode surface. Hence, the redox behaviour of HRP can be defined by this functional film. The data from this study show that such modified films can be useful for biological sensing and bioelectrochemical studies, while the immobilization of HRP on the riboflavin–nafion film exhibited good catalytic activity towards the reduction of H_2O_2. In view of the sensitivity, reproducibility and simplicity of the construction, this has great promise in the field of biosensors.

Acknowledgements

Financial supports from the University of Tehran and the Iranian National Science Foundation (INSF) are gratefully acknowledged. The authors also thank the Payam-e-Noor University Maybod, Yazd, Iran for arranging some of the equipment for the project.

References

Andrieux C P, Audebert P, Divisia-Blohorn B, Aldebert P and Michalak F 1990 Electrochemistry in hydrophobic nafion gels; *J. Electroanal. Chem.* **296** 117–139

Arvand M, Sohrabnezhad S, Mousavi M F, Shamsipur M and Zanjanchi M A 2003 Electrochemical study of methylene blue incorporated into mordenite type zeolite and its application for amperometric determination of ascorbic acid in real samples; *Anal. Chim. Acta* **491** 193–201

Cai X, Rivas G, Farias P A M, Shiraishi H, Wang J and Palecek E 1996 Evaluation of different carbon electrodes for stripping analysis of nucleic acids; *Electroanalysis* **8** 753–758

Chaubey A and Malhotra B D 2002 Mediated biosensors; *Biosens. Bioelectron.* **17** 441–456

Chen H Y, Ju H X and Xun Y G 1994 Methylene blue/perfluorosulfonated ionomer modified microcylinder carbon fiber electrode and its application for the determination of hemoglobin; *Anal. Chem.* **66** 4538–4542

Clarke M J, Harrison K L, Johnston K P and Howdle S M 1997 Water in carbon dioxide microemulsions: a spectroscopic study of a new environment for aqueous chemistry; *J. Am. Chem. Soc.* **119** 6399–6406

Gao Y, Li N, Zheng L, Zhao X, Zhang S, Han B and Ganzuo W H 2006 A cyclic voltammetric technique for the detection of micro-regions of bmimPF_6/Tween 20/H_2O microemulsions and their performance characterization by UV-vis spectroscopy; *Green Chem*. **8** 43–49

Gogol E V, Evtugyn G A, Marty J L, Budnikov H C and Winter V G 2000 Amperometric biosensors on nafion coated screen-printed electrodes for the determination of cholinesterase inhibitors; *Talanta* **53** 379–389

Gorton L, Karan H I, Hale P D, Inagaki T, Okamoto Y and Skothein T A 1991 An amperometric glucose electrode based on carbon paste, chemically modified with glucose dehydrogenase, nicotinamide adenine dinucleotide, and a phenoxazine mediator, coated with a poly(ester sulfonic acid) cation exchanger; *Anal. Chim. Acta* **249** 43–54

Honeychurch J and Rechnitz G A 1998 Voltammetry of adsorbed molecules. Part 2: irreversible redox systems; *Electroanalysis* **10** 453–457

Hong J, Ghourchian H, Rezaei-Zarchi S, Moosavi-Movahedi A A, Ahmadian S and Saboury A A 2007 Nafion–methylene blue functional membrane and its application in chemical- and bio-sensing; *Anal. Lett.* **40** 483–496

John S A and Ramaraj R 2004 Microenvironment effects on the electrochemical and photoelectrochemical properties of thionine loaded nafion films; *J. Electroanal. Chem.* **561** 119–126

Karyakin A A, Puganova E A, Budashov I A, Kurochkin I N, Karyakina E E, Levchenko V A, Matveyenko V N and Varfolomeyev S D 2004 Prussian blue based nanoelectrode arrays for H_2O_2 detection*; Anal. Chem.* **76** 474–478

Khoo S B and Chen F 2002 Studies of sol–gel ceramic film incorporating methylene blue on glassycarbon: an electrocatalytic system for the simultaneous determination of ascorbic and uric acids; *Anal. Chem*. **74** 5734–5741

Kong Y T, Boopathi M and Shim Y B 2003 Direct electrochemistry of horseradish peroxidase bonded on a conducting polymer modified glassy carbon electrode; *Biosens. Bioelectron.* **19** 227–232

Kubota L T, Gushikem Y, Perez J and Tanaka A A 1995 Electrochemical properties of iron phthalocyanine immobilized on titanium(IV) oxide coated on silica gel surface; *Langmuir* **11** 1009–1015

Kurzawa C, Hengstenberg A and Schuhmann W 2002 Immobilization method for the preparation of biosensors based on pH shift-induced deposition of biomolecule-containing polymer films; *Anal. Chem.* **74** 355–361

Laviron E 1982 Voltammetric methods for the study of adsorbed species; in *Electroanalytical chemistry* (ed.) A J Bard (New York: Dekker) p. 53

Liu H Y and Deng J Q1995 An amperometric lactate sensor employing tetrathiafulvalene in nafion film as electron shuttle; *Electrochim. Acta* **40** 1845–1849

McNeil C J, Greenough K R, Weeks P A and Cooper J M 1992 Electrochemical sensors for direct reagentless measurement of superoxide production by human neutrophils; *Free Radical Res. Commun.* **17** 399–406

Miura N, Kato H, Yamazoe N, Seiyama T and Kagaku D 1984 Amperometric gas sensor using solid-state proton conductor sensitive to hydrogen in air at room temperature; *Chem. Lett.* **11** 1905–1909

Munteanu F D, Kubota L T and Gorton L 2001 Effect of pH on the catalytic electro oxidation of NADH using different two-electron mediators immobilised on zirconium phosphate; *J. Electroanal. Chem.* **509** 2–10

Murray R W 1984 Chemically modified electrodes; in *Electro-analytical chemistry* (ed.) A J Bard (New York: Dekker) p. 191

Ogino Y, Takagi K, Kano K and Iketa T 1995 Reaction between diaphorase and quinone compounds in bioelectrocatalytic redox

reaction of NADH and NAD(+); *J. Electroanal. Chem*. **396** 517–524

Pessoa C A, Gushikem Y, Kubota L T and Gorton L 1997 Preliminary electrochemical study of phenothiazines and phenoxazines immobilized on zirconium phosphate; *J. Electroanal. Chem.* **431** 23–27

Richard P B and Erno L 1994 Recomendations for nomenclature of ion-selective electrodes; *IUPAC* **66** 2527–2536

Rolison D R 1990 Zeolite-modified electrodes and electrode-modified zeolites; *Chem. Rev.* **90** 867-878

Scheller F W, Bistolas N, Liu S, Jänchen M, Katterle M and Wollenberger U 2005 Thirty years of haemoglobin electrochemistry; *Adv. Coll. Interf. Sci.* **116** 111–120

Tian Y, Ariga T, Takashima N and Okajima T 2004 Self-assembled monolayer suitable for electron transfer promotion of copper, zinc-superoxide dismutase*; Electrochem. Comm.* **6** 609–614

Yamashita M, Rosatto S S and Kubota L T 2002 Electrochemical comparative study of riboflavin, FMN and FAD immobilized on the silica gel modified with zirconium oxide; *J. Braz. Chem. Soc*. **13** 635–641

Yao H, Li N, Xu S, Xu J Z, Zhu J J and Chen H Y 2005 Electrochemical study of a new methylene blue/silicon oxide nanocomposition mediator and its application for stable biosensor of hydrogen peroxide; *Biosens. Bioelectron.* **21** 372–377

MS received 20 September 2007; accepted 15 February 2008

*e*Publication: 4 April 2008

Corresponding editor: Shahid Khan