Characterization and electrochemical study of nano-composition based methylene blue- and riboflavin-nafion on the surface of a gold electrode

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A functional membrane, composed of the nanoparticles of methylene blue (MB) and Nafion, was constructed during the present study. The materials were characterized by the methods of scanning electron microscopy (SEM), transmission electron microscopy (TEM), and UV-Visible and FT-IR. The average diameter of new nano-particles was estimated to be about 60 nm. A novel Nafion-riboflavin membrane was also constructed and characterized by the methods of SEM, TEM and UV-Visible spectroscopy. The estimated average diameter of the new nanoparticles was about 60 nm. The functional membranes of Nafion-riboflavin and Nafion-methylene blue showed a quasi-reversible electrochemical behavior, on the gold electrode, with a formal potential of –562 $\pm$ 5 and –305 $\pm$ 5 mV (vs. Ag/AgCl), respectively. Some electrochemical parameters were also estimated, indicating that the systems present good and stable electron transfer properties. Our data proved that Nafion can be an interesting and helpful material in constructing nanoparticles of different electro-active materials and in their stable immobilization.

**Key Words:** Riboflavin, methylene blue, Nafion, electrode, characterization, nanoparticles.

Introduction

The immobilization of electron mediators onto the electrode surfaces, to produce chemically modified electrodes for use in electro-analysis, has been widely investigated during the last 2 decades.[1−4] Electrodes modified

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with quinones, flavins, and other quinoic type compounds, such as phenoxazines, have been widely exploited for sensor development.[5−8] The importance of flavins in enzymatic catalysis has motivated electrochemical behavior studies of the monolayers or aggregates, containing the synthetic flavins.[9] The flavin nucleotide has a unique ability to transfer 1 or 2 electrons and to promote easy reduction of the molecular oxygen.[10] Flavins also work as the redox coenzymes in many biological transformations and, hence, they have been used as redox mediators between the electrode and several enzymes.[11] In addition, riboflavin (RF) is an efficient mediator for the bioelectro-chemical oxidation of nicotinamide adenine dinucleotide (NADH).[12] Thus, there is special interest in the study of flavin-modified electrodes for electrochemistry and bio-electrochemical investigations.[13−15] A series of organic dyes, such as methylene blue (MB), methylene green, Prussian blue, phenazines, and thionin, have been used as electron transfer mediators.[16−20] Among them, MB is a phenothiazine dye with a planar structure that forms a deep blue solution when dissolved in water.[21,22] This cationic dye, whose electrochemical properties are well known in the solution phase, has been used as a redox indicator since its formal potential ($E^{\circ\prime}$) is between –0.25 and 0.08 V (versus SCE) at the pH range of 2.0-8.0. However, such a low molecular-weight soluble mediator is disadvantageous as it can leach out of the electrode, which may lead to a significant loss of signal and affect the sensor's stability. Yet Chen et al. reported that no change in peak currents was recorded when MB/Nafion-modified carbon fiber micro-cylinder electrode was kept in buffer solution for 1 week, indicating the lack of the dissolution of MB molecules from the polymer film into the aqueous solution.[23,24] Nafion is a perfluorinated anionic polyelectrolyte.[24] The increasing popularity of Nafion for the fabrication of redox polymer modified electrodes in recent years arises from the easy fabrication, good electrical conductivity, and high partition coefficients of many redox compounds in Nafion. In addition, the Nafion film has high chemical stability, good biocompatibility, and the ability to resist interference from anions and biological macromolecules.[25−27] The chemical structure of Nafion is schematized in Figure 1. Here, we report the development of 2 new nanoparticle-based functional membranes with very good stability on the gold electrode. The first membrane was composed of Nafion and MB while the other was composed of Nafion and RF.
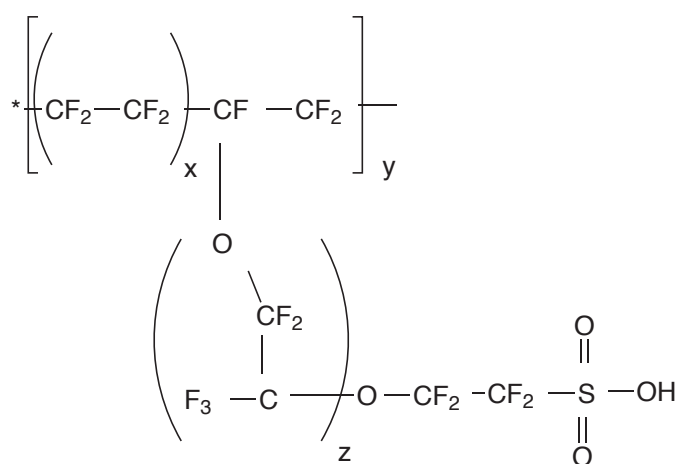
Figure 1. Schematic structure of Nafion.

Experimental

Reagents

Methylene blue (MB) and NaOH were purchased from Merck. Riboflavin (RF), Nafion (perfluorosulfonated ion-exchange resin, 5% ethanol solution) and 3-N-Morpholino propanesulfonic acid (MOPS) were obtained from Sigma. The solutions were prepared in de-ionized double distilled water (18 MΩ cm, Barnstead Instruments).

Apparatus and measurements

UV-Visible absorption spectra were obtained with a Cary 100 Bio (Varian, Australia). The SEM and TEM images were captured with DSM 960A and CEM 902A (Zeiss, Germany), respectively. Fourier transform infrared (FT-IR) spectra, by KBr pellets, were obtained, in the range of 400-2000 cm^{-1}, on an FTIR 4300 (Shimadzu, Japan) spectrometer at room temperature.

Methylene blue- and riboflavin-Nafion modified gold electrode

For the preparation of the Nafion-RF 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 electrochemically treated in 0.2 M sulfuric acid, cycling between –0.2 and +1.5 V (vs. Ag/AgCl) at a sweep rate of 0.1 V s^{-1}, until the appearance of a clean gold electrode was obtained. Finally, the electrode was washed with de-ionized 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 RF solution (1 mM) for 10 min, washed carefully with de-ionized double distilled water, and stored at 4 °C when not in use.

The experimental solutions were de-aerated using the highly pure nitrogen for 30 min and a nitrogen atmosphere was kept over the solutions during the whole measurement. All of the electrochemical measurements were carried out in 0.1 M MOPS buffer solution (pH 7.0) at 25 ± 1 °C. The same procedure was repeated for the construction of the Nafion-MB modified electrode.

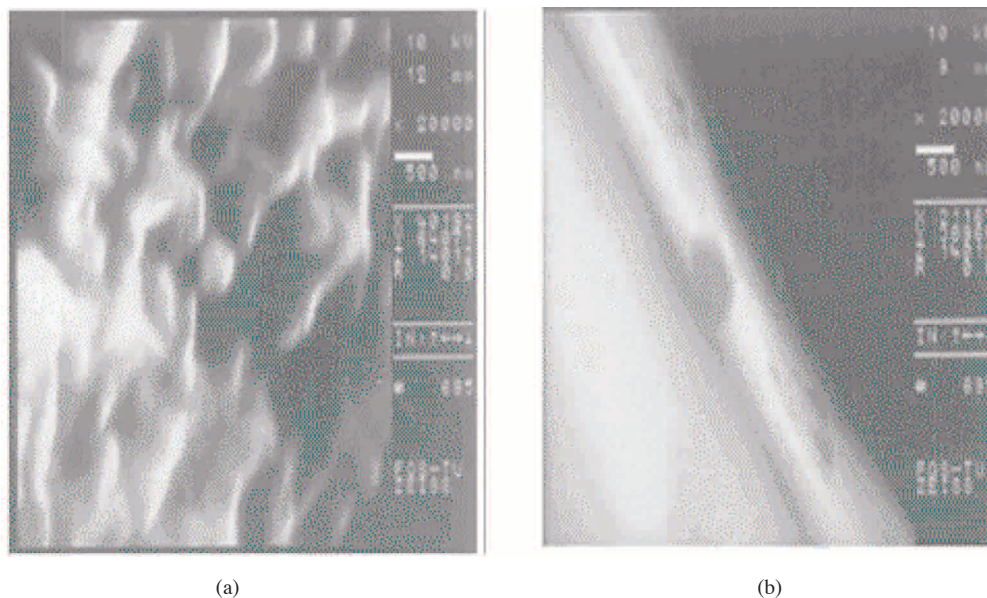## Electron microscopy of the membranes

The TEM images of Nafion-MB particles were prepared by the following method: at first, a solution, with the same volumes of 1 mM MB and 5% Nafion, was prepared and diluted 100 times with 50% ethanol. A drop of the diluted solution was added to fomvar/carbon coated grids (400 meshes) and after drying, viewed under TEM operating at 80 kV. The same procedure was done in the case of the nafion-RF.

Results and discussion

Electron microscopic images

The Nafion-MB and Nafion-RF surfaces were viewed under SEM and TEM to consider the uniformity and other characteristics of the membranes. The cross-sectional images of the membranes, taken by SEM, represented the

interface between the substrate and membranes. It could be seen that the membranes had a good contact with substrate, with thickness of about 800-1200 nm (Figure 2a). The top view of the Nafion membrane showed a lot of meshy protuberances and hollow structures (Figure 2b). The TEM images showed that the Nafion-MB (Figure 3a and b) and Nafion-RF (Figure 3c and d) particles were in a mixture of integrated and single particle form, made from uniform spheres with the average particle size of about 60 nm.

(a)   (b)

**Figure 2.** Scanning electron micrographs of Nafion membrane: (a) cross section, (b) top view.

Spectroscopic studies

In fact, the MB and RF have potential to be strongly immobilized onto the electrode surface, and their modified electrodes would not only be stable but also, according to Chen et al., these nanoparticle structures would greatly increase their surface area.[28] On the other hand, MB molecules would homogeneously disperse inside the particles.[29]
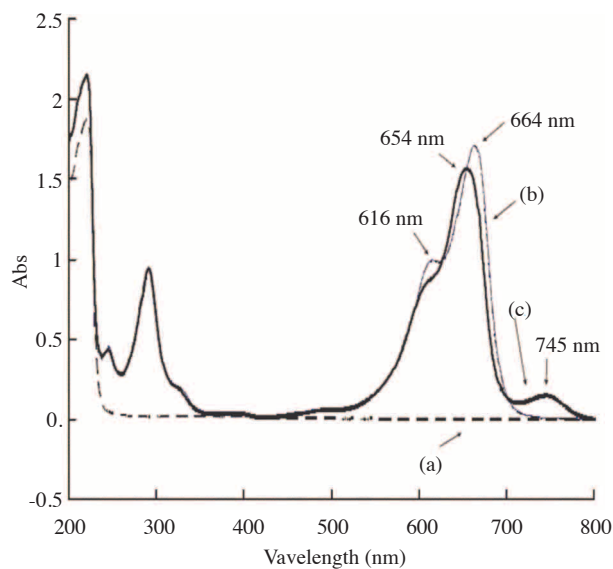
Figure 4 (a, b, and c) shows the UV-Visible spectra of the MB, Nafion, and their combination (Nafion-MB), respectively. By adding MB to the Nafion, while no change was observed in the MB peaks at 250 and 280 nm, the absorption peaks had a significant change at around 664 and 616 nm. According to Ong et al., the prominent peak, at around 664 nm, indicates the monomer form, whereas the hump, at around 616 nm, designates the dimer form of MB.[30] By exposing the MB-Nafion membrane to air, the MB might be changed to oxidized positive form at the surface of the membrane.[30] This process facilitates the electrostatic interaction between the positively charged MB and the negatively charged Nafion, which led to a small blue shift (10 nm) at around 664 nm. Meanwhile, the appearance of a new peak at 746 nm could be a sign of the formation of a new MB derivative (Figure 4c).

Figure 5 shows the UV-visible spectra of riboflavin adsorbed onto the Nafion (a) and in solution (b). 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 considerably. As described before, micro-emulsion and nanoparticle production of

materials decreases their absorbance in UV-Visible region.[31−33] Hence, the results determine that the Nafion particles gather around some of the riboflavin molecules and develop nanoparticles. The Nafion structure has an abundance of F and O atoms, which can develop very strong hydrogen bonding with some H atoms contained in riboflavin molecules. The data, obtained by electron microscopy in the present study, show the construction of nanoparticles while the UV-Visible spectra certify these findings.
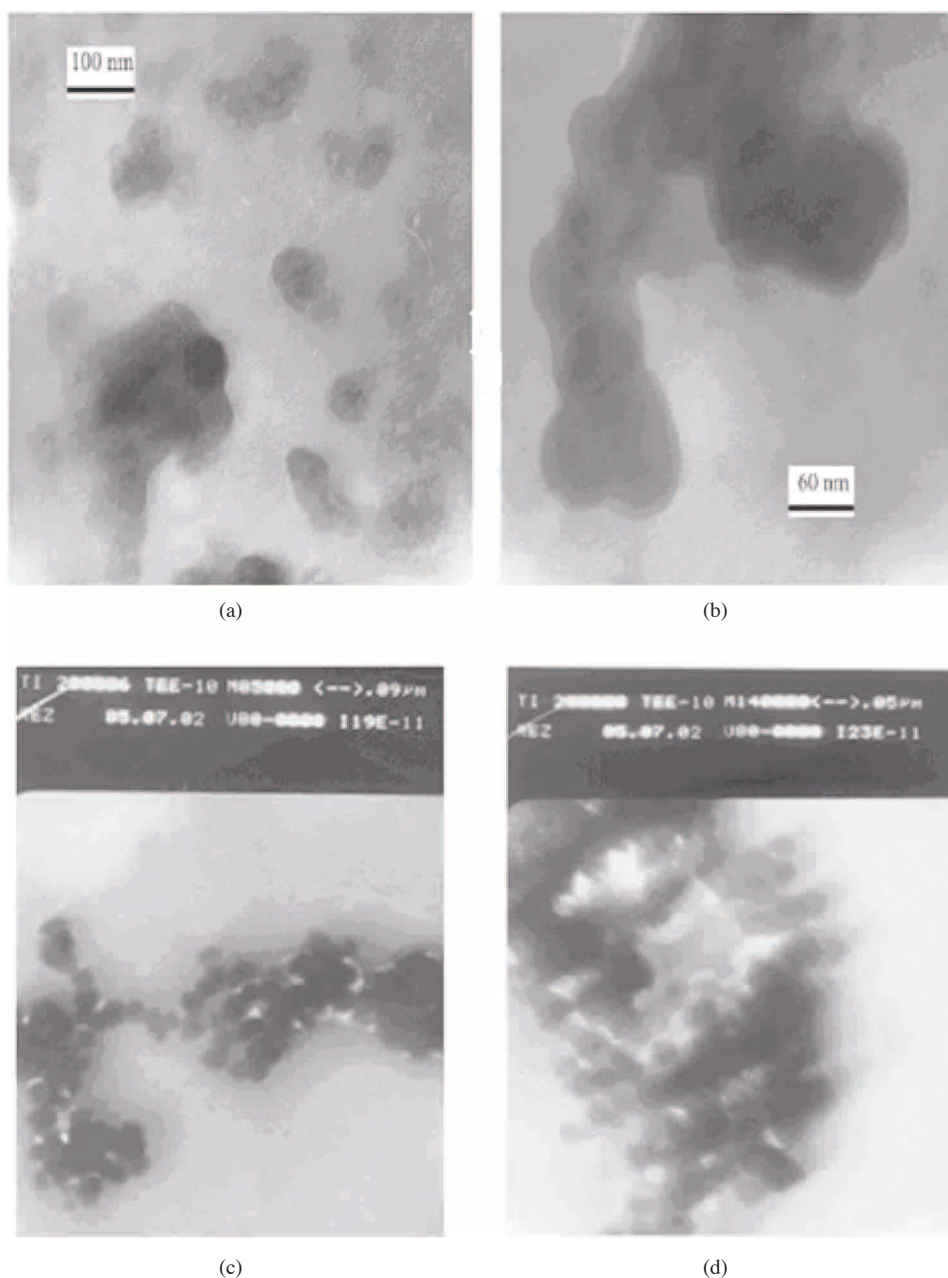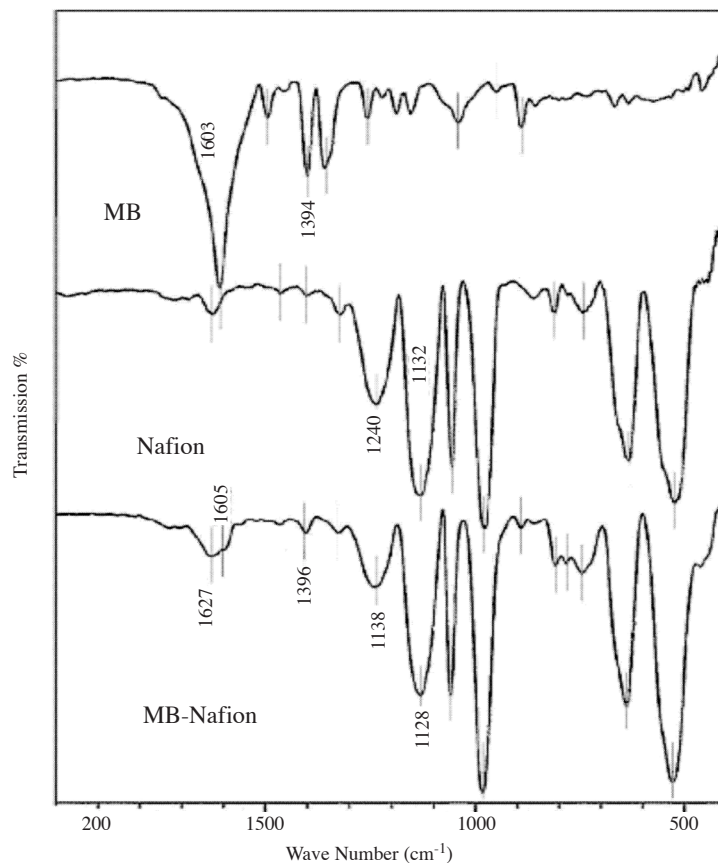

Figure 3. TEM images (a, b) of Nafion-MB and (c, d) Nafion-RF particles.

Figure 4. The UV-Visible spectra of Nafion (a), MB (b) and Nafion-MB (c).
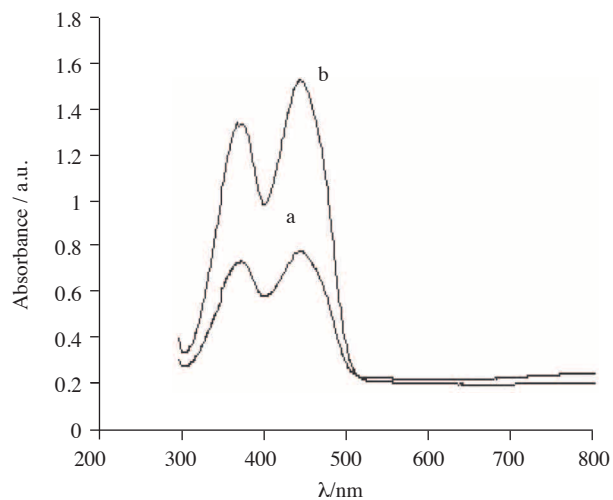
Figure 5. UV-Visible spectra of riboflavin adsorbed on Nafion (a) and in solution (b).

**Figure 6.** FT-IR spectra of MB, Nafion and Nafion-MB.

Figure 6 shows the FT-IR spectra of the MB, Nafion, and a combination of MB-Nafion. The characteristic peaks of the $-SO^{-3}$ group of Nafion at $\sim$1240 and $\sim$1132 cm^{-1}, and of the aromatic ring of MB at $\sim$1603 and $\sim$1394 cm^{-1} appeared in the Nafion-MB combination.[34,35] However, some small shifts were also observed. The MB absorption band at 1603 cm^{-1}, corresponding to the vibration of the aromatic ring, shifted to 1605 cm^{-1}. Moreover, the adsorption band of Nafion at $\sim$1240 cm^{-1}, attributed to $-SO^{-3}$ asymmetric stretch, shifted to $\sim$1238 and that at $\sim$1132 cm^{-1}, attributed to $-SO^{-3}$ symmetric stretch, shifted to $\sim$1128 cm^{-1}. These data indicate the bonding interaction between the $-SO^{-3}$ of Nafion and the aromatic ring of MB. However, Watanabe et al. emphasized the bond formation between the $-SO^{-3}$ of Nafion and nitrogen of the aromatic ring of MB.[36]

## Electrochemical properties of the Riboflavin-Nafion modified gold electrode

Cyclic voltammograms obtained for the Nafion-modified gold electrode did not show any peak current in the investigated potential range, while in the case of the Nafion-riboflavin modified gold electrode, a clear redox peak was scanned that indicates riboflavin immobilization on the Nafion. This observation represents a good electrochemical signal for the recent analysis. The formal potential ($E^{\circ'}$), determined by using the following equation: $E^{\circ'} = (E_{pc} + E_{pa})/2$ (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 anodic and cathodic peak currents on the square root of the scan rates ($v^{1/2}$) is illustrated in Figure 7 for the adsorbed riboflavin. This behavior is similar to a diffusion-controlled redox process but no decrease in the peak current was observed after repeated cycles of this experiment. These findings indicate that the riboflavin is strongly adsorbed onto the Nafion surface. Therefore, the redox process is controlled by the diffusion of counter-ions to keep the electro-neutrality on the electrode surface. Other possibilities of this behavior can be due to the resistance of the material or an electron transfer process, occurring by the electro-hopping mechanism,[37] since this behavior is more likely to be seen when the electron transfer mechanism occurs at 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 the changes in scan rate. In addition, the ratios between the cathodic and anodic peak currents were near unity. This behavior shows a good electron transfer rate between the adsorbed riboflavin and the electrode surface.[38,39]

Electrochemical properties of the Methylene blue-Nafion modified gold electrode

Figure 8 represents the cyclic voltammograms of MB-Nafion on the surface of the electrode at different scan rates in 0.05 M MOPS buffer, pH 7.0, at 25 °C. Figure 8 (Inset A) shows that in the range of scan rate (v) from 20 to 400 mV/s both the anodic and cathodic peak currents are proportional to v. There was no redox peak for Nafion membrane in the absence of MB (Figure 8, inset B). Nafion-MB membrane was immobilized on the gold electrode. The current ratio of the anodic/cathodic peak currents at different scan rates are close to 1 (-Ipa/Ipc = 2.22/2.25 = 0.99 at 100 mV/s) and the separation between the anodic and cathodic peak potentials (XEp = Epa-Epc) is estimated to be 110 mV at 100 mV/s. These indicate that the electrochemical process of Nafion-MB functional membrane is quasi-reversible at the gold electrode. Moreover, the formal potential, E°', estimated as (Epa + Epc)/2, was –305 ± 5 mV vs. Ag/AgCl in 0.05 M MOPS buffer pH 7.0 at 25 °C. This redox potential is close to that reported in the literature.[40]
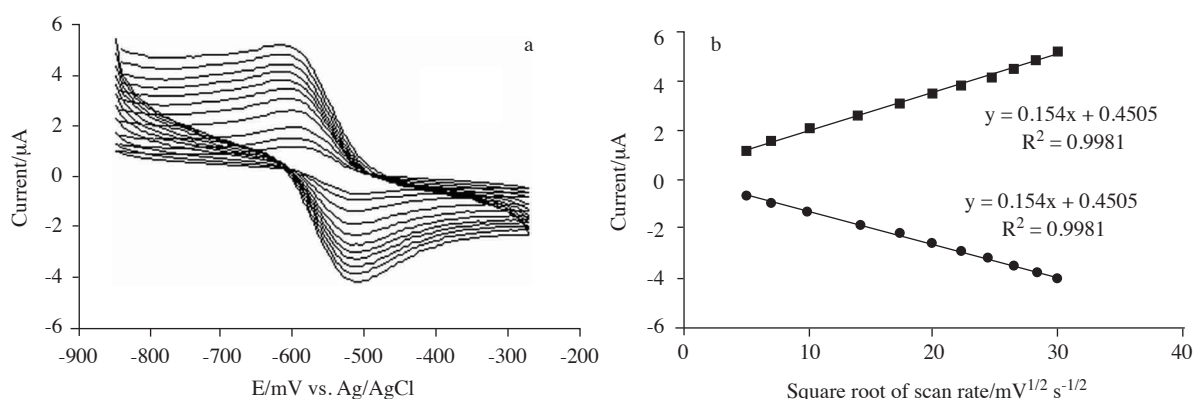
Figure 7. (a): Typical cyclic voltammograms of Nafion-riboflavin on the gold electrode at different scan rates. The voltammograms (from inner to outer) designate the scan rates of 25, 50,100, 200, 300, 400, 500, 600, 700, 800, 900, and 1000 mV/s, respectively. (b): Dependence of the anodic and cathodic 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} MOPS buffer solution.
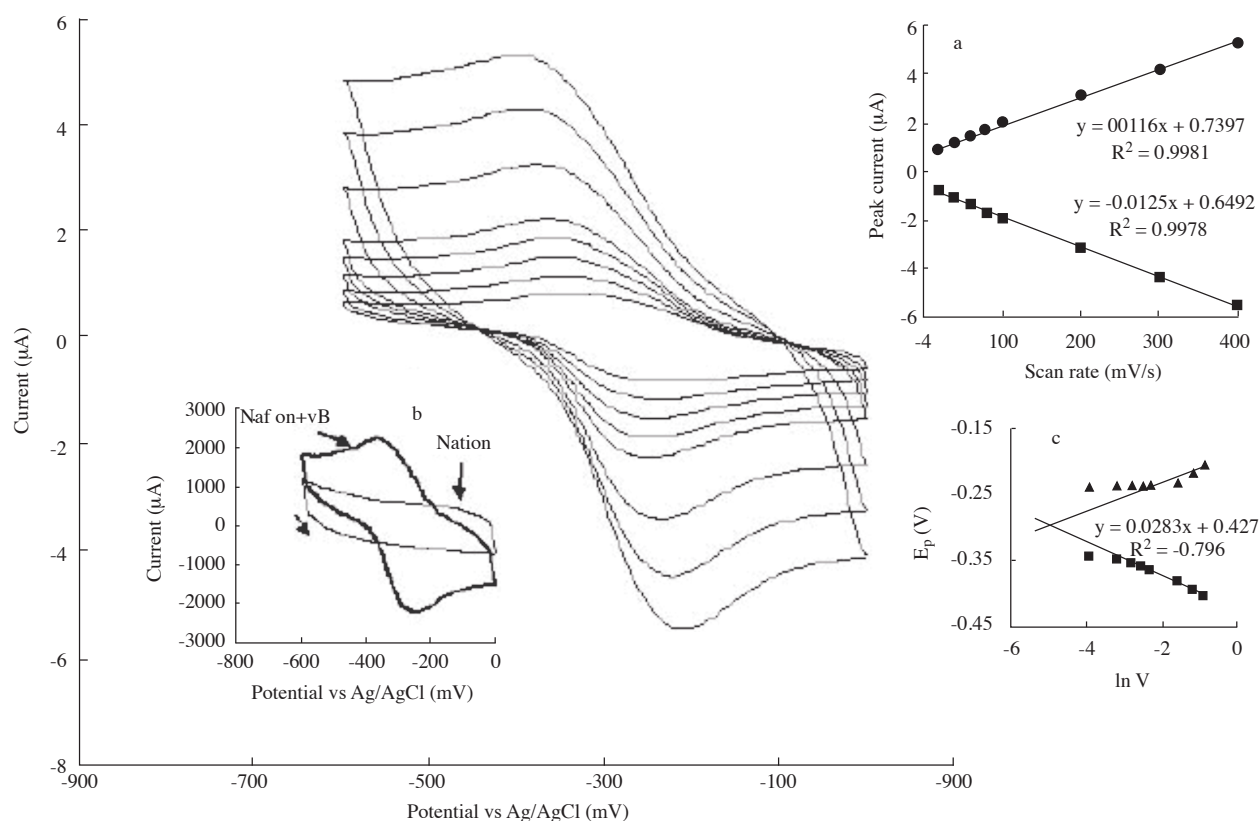
Figure 8. Typical cyclic voltammograms (CVs) of MB-Nafion on the gold electrode at different scan rates. The voltammograms (from inner to outer) designate the scan rates of 20, 40, 60, 80, 100, 200, 300, and 400 mV/s in the pH 7.0, 0.05 M MOPS buffer, respectively. Insets: (A) Peak current dependence on scan rate, (B) CV of Nafion and Nafion-MB modified electrode and (C) peak potential (Ep) dependence on ln$v$.

Conclusions

The present study has introduced 2 functional membranes, composed of nano-particles of MB-Nafion and RF-Nafion. The spectroscopic studies have shown that this poly-organic nano-composite has properties different from either MB or Nafion and either RF or Nafion. The results have also shown that Nafion was able to immobilize the RF and MB onto the electrode surface with good stability and facilitated electron transfer between RF and MB, immobilized onto the electrode surface, by Nafion.

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