



## Characterization Study for Nanocompositions of Methylene Blue and Riboflavin-Nafion on the Electrode Surface

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### Abstract

Nafion is a perfluorinated anionic polyelectrolyte. The increasing popularity of nafion for the fabrication of redox polymer modified electrodes in recent years arises from easy fabrication, good electrical conductivity and high partition coefficients of many redox compounds in nafion. To investigate the production of nano-compositions by mixing electron transfer material and nafion polymer for the modification of electrodes, a functional membrane composed of nano-particles of methylene blue, and nafion was constructed. The materials were characterized by the methods of scanning electron microscopy (SEM), transmission electron microscopy (TEM), ultraviolet (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 new nanoparticles was about 60 nm. Our data has proven that nafion can be very interesting and helpful material in constructing nanoparticles of different electro-active materials and it can immobilize this material with a very good stability.

*Keywords:* Functional membrane; Methylene blue; Nafion; Nanoparticles; Riboflavin.

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### 1. Introduction

Nafion is a perfluorinated anionic polyelectrolyte [1]. The increasing popularity of nafion for the fabrication of redox polymer modified electrodes in recent years arises from 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 also ability to resist interferences from anions and biological macromolecules [2-4]. Chemical structure of nafion could be schematized in Figure 1. During recent years, numerous investigations have been carried out on electrochemical biosensors for advancement of the rapidity, selectivity, sensitivity and low cost of chemical analysis [5-7]. The

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immobilization of electron transfer mediators onto the surface of electrodes has been widely investigated during the last two decades. These mediators are used to produce chemically modified electrodes for electrochemical analyses [8-11]. The nafion immobilization process is simple and versatile, which offers good retention of the biochemical and recognition properties, increasing the stability and can also improve the electrochemical properties of the immobilized species. Considering these aspects, present work describes two new types of functional membranes composed of nanoparticles. The first membrane was composed of nafion and methylene blue (MB) and the second was composed of nafion and riboflavin.

## 2. Materials and methods

### 2.1. Reagents

Methylene blue 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 deionized double distilled water (18 M $\Omega$  cm, Barnstead Instrument).

### 2.2. Apparatus and measurements

Ultraviolet (UV)-visible absorption spectra

were obtained with Carry 100 Bio (Varian, Australia). The images for scanning electron microscope (SEM) and transmission electron microscope (TEM) were obtained 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<sup>-1</sup> on an FT-IR 4300 (Shimadzu, Japan) spectrometer at room temperature.

### 2.3. Electron microscopy of the membranes

For preparing samples of SEM images, 5  $\mu$ l of the mixture of the same volume of 5% nafion and 1 mM MB was dropped onto the surface of the electrode. After drying the electrode surface in air for 2 h, it was ready. For preparing nafion-RF modified gold electrode, we did the same method. The TEM images of nafion-MB and nafion-RF 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 this diluted solution was added to fomvar/carbon coated grids (400 meshes) and after drying, viewed under TEM operating at 80 kV. For nafion-MB we did the same method.

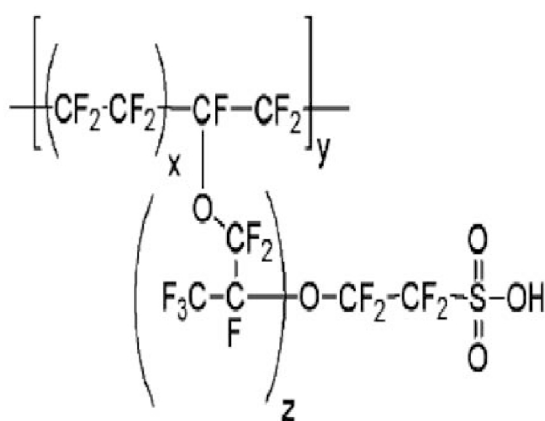


Figure 1. Schematic structure of nafion.

### 3. Results and discussion

#### 3.1. Electron microscopic images

The nafion-MB and nafion-RF surfaces were viewed with SEM and TEM to consider the uniformity and other characteristics of the membranes. The cross sectional images of the membranes, taken by SEM, represent the interface between substrate and membranes.

It could be seen that the membranes were contacted with the substrate well and had a thickness of about 800-1200 nm (Figure 2a). Top view of the nafion membrane showed lots of meshy protuberance and hollow structures (Figure 2b). The TEM images showed that the nafion-MB (Figure 3a and 3b) and nafion-RF (Figure 3c and 3d) particles were in a mixture of integrated and single particle form, made from the uniform spheres with the average particle size of about 60 nm.

#### 3.2. Spectroscopic studies

In fact, the MB and RF have a potential to be immobilized strongly on the electrode surface and their modified electrodes would not only be stable but also according to Chen *et al.* [12], these nanoparticle structures would greatly increase the surface area. On the other hand, MB molecules would homogeneously disperse inside the particles [13].

Figure 4 shows the UV-visible spectra of the MB, nafion and their combination (MB-nafion), respectively. By adding MB to nafion, while no change was observed in the MB peaks at 250 and 280 nm, the absorption peaks at around 664 and 616 nm were changed, significantly. According to Ong *et al.* [14], the prominent peak at around 664 nm indicates the monomer form whereas the hump at around 616 designates the dimer form of MB. By exposing the MB-nafion membrane to air, at the surface of membrane, the MB might be changed to oxidized positive form (oxidized and reduced state of MB are shown in Figure 1B) [14]. This process facilitates the electrostatic interaction between positively charged MB and the negatively charged nafion, which led to a small blue shift (10 nm) at around 664 nm. Meanwhile, appearance of a new peak at 746 nm could be a sign of formation of the new derivative of MB.

Figure 5 shows the UV-visible spectra of riboflavin adsorbed onto the (a) nafion, and (b) solution, respectively. By adding RF to nafion, no changes were observed on the status of RF peaks at 364 and 440 nm but the adsorption was decreased up to considerable levels. As described before, micro-emulsion

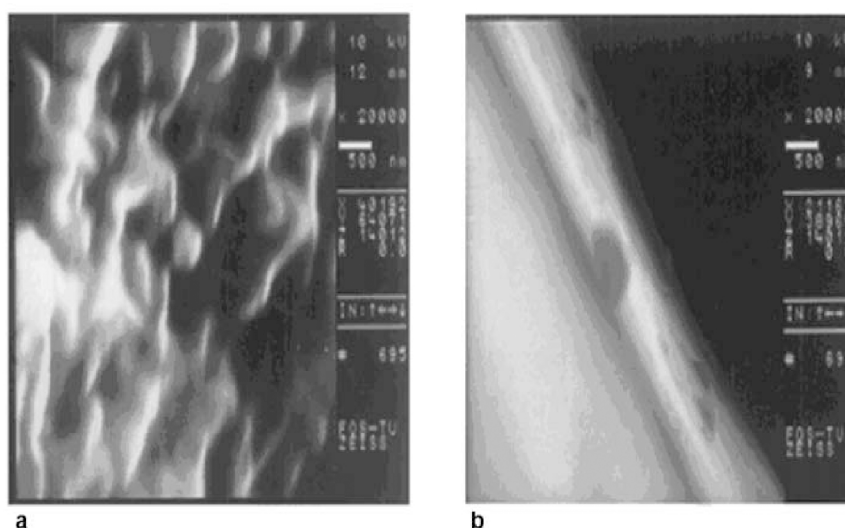
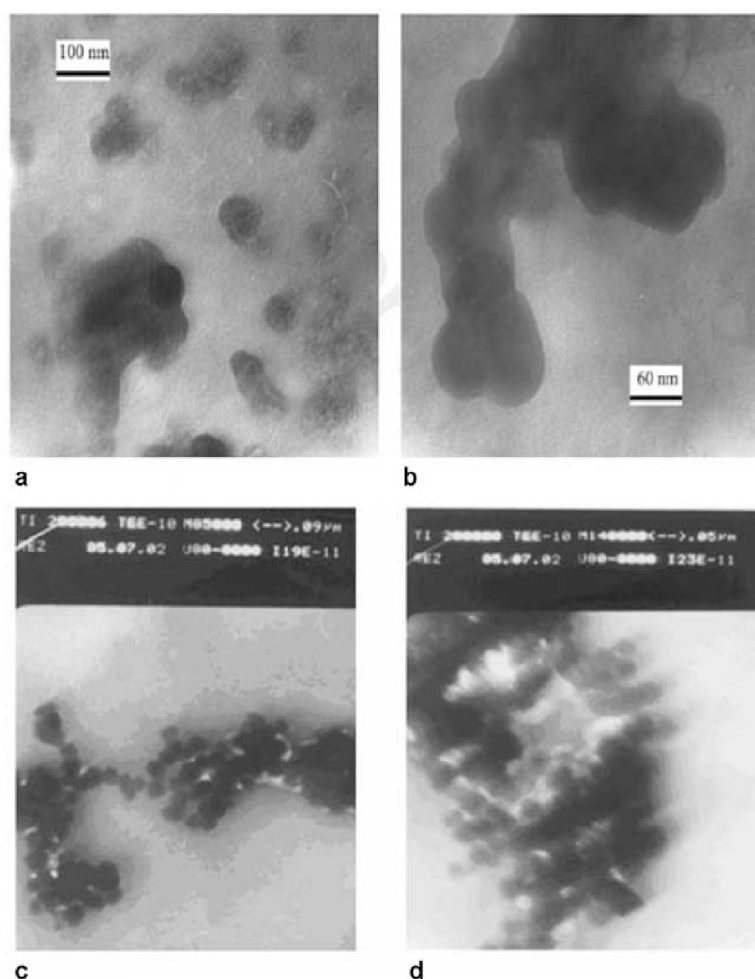


Figure 2. Scanning electron micrographs of nafion membrane: (a) cross section, (b) top view.

and nanoparticle production of materials decreases their absorbance in UV-visible region [15-17]. Hence, the results determine that the nafion particles gather around some of the RF molecules and develop nanoparticles. The nafion structure has an abundance of F and O atoms, which can develop very strong hydrogen bonds with some H atoms contained in RF 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 6 shows the FT-IR spectra of the MB, nafion and a combination of MB-nafion. The characteristic peaks of  $-\text{SO}_3^-$  group of

nafion at  $\sim 1240$  and  $\sim 1132$   $\text{cm}^{-1}$ , and of aromatic ring of MB at  $\sim 1603$  and  $\sim 1394$   $\text{cm}^{-1}$  appeared in the nafion-MB combination [18, 19]. However, some small shifts were also observed. The MB absorption band at  $1603$   $\text{cm}^{-1}$ , corresponding to the vibration of the aromatic ring, shifted to  $1605$   $\text{cm}^{-1}$ . Moreover, the adsorption band of nafion at  $\sim 1240$   $\text{cm}^{-1}$ , attributed to  $-\text{SO}_3^-$  asymmetric stretch, shifted to  $\sim 1238$  and that at  $\sim 1132$   $\text{cm}^{-1}$ , attributed to  $-\text{SO}_3^-$  symmetric stretch, shifted to  $\sim 1128$   $\text{cm}^{-1}$ . These data indicate the bonding interaction between the  $-\text{SO}_3^-$  of nafion and the aromatic ring of MB. However, Watanabe *et al.* [20] emphasized on the bond formation between the  $-\text{SO}_3^-$  of nafion and nitrogen of the aromatic ring of MB.



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

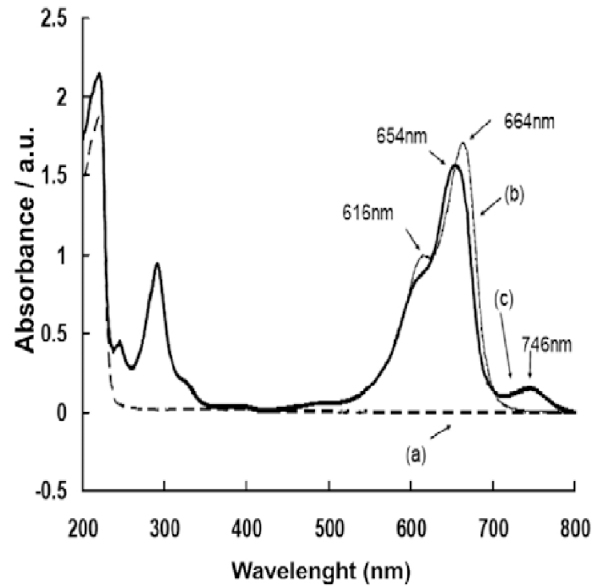


Figure 4. The UV-visible spectra of nafion (a), MB (b) and nafion-MB (c).

#### 4. Conclusions

A functional membrane composed of nanoparticles of MB and nafion was constructed. The spectroscopic studies showed that this poly-organic nano-composite represents different properties from either MB or nafion. The results also described that nafion material

was able to immobilize the BF onto the surface of electrode with good stability. TEM and UV-visible spectroscopic analyses showed the construction of RF nanoparticles on to the nafion polymer.

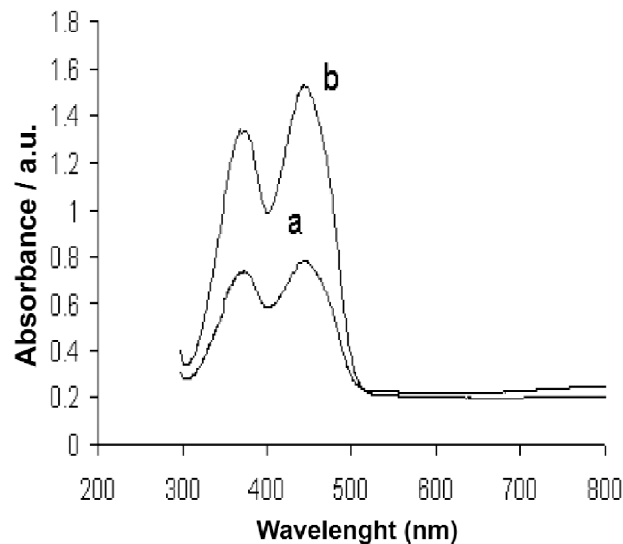


Figure 5. UV-visible spectra of riboflavin adsorbed on nafion (a) and in solution (b).

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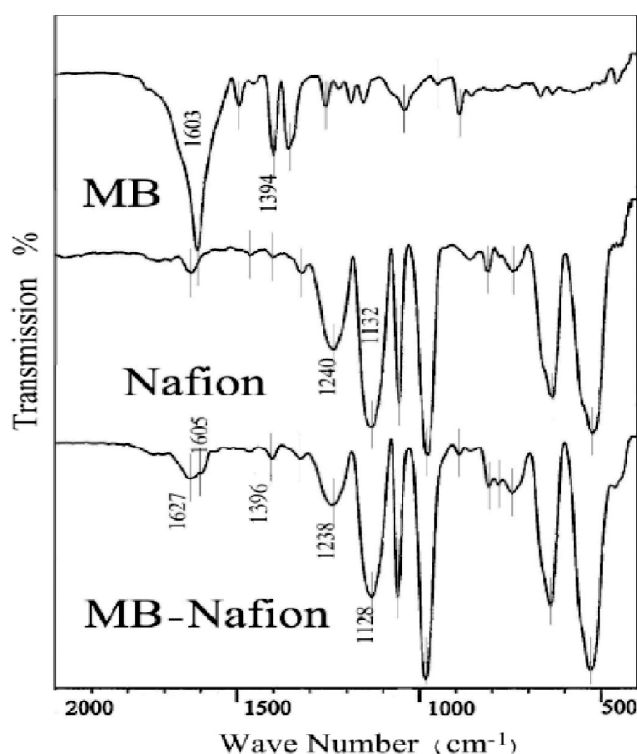


Figure 6. FTIR spectra of MB, nafion and nafion-MB.

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