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Potentiometric Detection of K^+ Ions Using Gramicidin-Ionophore/Lipid Bilayer Assembly on Polyaniline Electrode

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Summary: Organic electronics have their advantage over traditional silicon or inorganic electrodes in their easy processing and flexibility. For instance, polyaniline has shown to be promising electrode material for various sensing opportunities including as a use of pH-meter. As the ion-to-electrode transduction may be optimal in these organic materials with high surface area and controllable electric double layer, selectivity has often been approached with the use of ionophores. We have inserted a gramicidin ionophore in a lipid bilayer assembled on polyaniline surface and measured successfully K^+ ion concentration potentiometrically.

Keywords: gramicidin; ion selective electrode; lipid bilayer; polyaniline

Introduction

In the quest of scientists to develop materials with great potentiality, polyaniline becomes widely investigated and used conducting polymer not just because of its intrinsic properties, but more for the various possibilities to functionalize this polymer to unique and applicable materials in the design of electronic, micro-electronic devices, rechargeable batteries and sensors. Polyaniline films are being increasingly used in the design of bio-sensors, actuators, receptors and transducers because of the exceptional properties exhibited by such films. The electrical conductivity of conducting polymers changes over several orders of magnitudes in response to change in pH.[1] Lately, considerable efforts have been directed towards the development of PANI films sensors. As an example, copolymer film of PANI and poly(3-aminophenylboronic acid) was used for reversible detection of saccharides.[2] As the ion-to-electron transduction properties of PANI may be optimal, the selectivity factors in sensing can only be obtained by further modification of PANI.

One of the most recent approaches to functionalize polyaniline (PANI) is based on the novel concurrent reduction and substitution chemistry with electron rich atoms or groups usually called nucleophiles. This approach has been thoroughly investigated and reported by C.-Chung Han et al[3–5] and Y. Zhou et al[6] as an effective manner to reduce the polyaniline backbone while derivatizing it. In this novel chemistry, the nucleophile is reported to attack the quinoid ring of the emeraldine base and convert it to a functionalized leucoemeraldine containing only secondary amines. This modified form of PANI can be reoxidized to regenerate the quinoid rings that provide active sites for further cycles of reduction and functionalization. The bond formation occurs between the electron donating atom (S) of the thiol and the reactive diiminoquinoid ring of polyaniline. The thiol therefore, reduces the polyaniline backbone while concurrently functionalizing it. As a result of this reduction, the strained and less stable quinoid ring is converted to a more stable benzenoid ring.

More specific functionalization can be done by adding metal complexes, chemical

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agents, various organic species, enzymes, antibodies, peptides etc, as reported by Bergman and Hanks.[13] The incorporation of moieties into the a conducting polymer matrix can be done by a variety of techniques: 1) non-covalent adsorption of enzyme done in aqueous phase 2) deposition by Langmuir-Blodgett technique where highly ordered films with receptors are obtained; 3) layer-by-layer deposition with electrostatic doping of polymers, 4) electropolymerization of PANI in a solution containing both the monomer and the additive of interest; and/or 5) covalent immobilization by reacting the polymer film with the biomolecule or chemical of interest.

Functionalized PANI is not a stand-alone film due to weak mechanical properties and thus needs to be applied on a solid substrate. The growth of polyaniline film on several solid supports has been and continued to be extensively investigated.[7–11] However, it was first noted in 1989 by McDiarmid et al that PANI deposited by in situ adsorption polymerization to form strongly adhering films on a variety of substrates.[12] The following substrates have been used to grow PANI films: ITO (indium tin oxide), silicon dioxide, titanium dioxide, glass etc. Since in this research platinum electrode is used, it worth describing the electrochemical characteristics of the platinum surface electrodes.

Electrochemical detection provide an attractive means of synthesizing material and measuring samples responses due to direct conversion of the redox reaction taking place in the cell to a potential reading. The most common techniques are cyclic voltammetry, chronopotentiometry, chronoamperometry, potentiometry, and impedance spectroscopy. In electrochemical sensing technique, three electrodes cell setup is required: a reference electrode, a counter electrode and a working electrode. The reference electrode commonly used is the silver-silver chloride electrode must be kept at a distance from the reaction site so its known potential is maintained stable. The working electrode serves as a transduction element in the biochemical reaction, while the counter electrode establishes a connection to the electrolytic solution so that a current can be applied to the working electrode. All three electrodes must be conductive and chemically stable. Electrodes such as platinum, gold, glassy carbon, silicon are the most commonly used. Overall, electrodes are the heart of any reaction carried out in an electrochemical cell therefore they must be in good conditions in order to capture proper and reliable detection.

Langmuir-Blodgett deposition method (LB) is used to transfer Langmuir monolayers of amphiphilic molecules onto hydrophilic substrate such as glass and quartz[14] semi-conductors,[15–17] mica[18,19] and oxidized metal surfaces.[20] LB method is also effective for transferring monolayers on hydrophobic substrates such as hydrogen terminated or silanized Si[21,22] or silanized glass.[23] Several investigations of gramicidin films at the air water interface have been conducted. One study carried out by Mau et al[24] reported the molecular area, the surface pressure and the surface potential of various forms of gramicidin films in the absence of lipid. The findings of this study revealed that the molecules are aligned parallel to the water surface either in the monomeric or the double helical conformation. The reported area per molecule before collapse reported for Gramicidin A varies from 130–150[25–27] to 250 Å^2/molecule.[24] However, there has not yet been any report of membrane incorporated gramicidin study supported on polyaniline film.

We report here the investigation of PANI-alkylthiol reaction in solution via spectroscopic methods in order to develop a thorough understanding of the mechanism. Based upon the information gathered from this work, electrochemically made polyaniline films are further used as a matrix to anchor a bilayer-peptide membrane.

Experimental Part

Reagents

Ammonium peroxydisulfate, hydrochloric acid, potassium phosphate monobasic

(KH_2PO_4), potassium hydroxide (KOH), phosphoric acid, ammonium hydroxide, 1,4-dioxane, tetrahydrofuran, acetone, potassium chloride, grade 99%, and concentrated sulfuric acid were all purchased from Baker. Aniline hydrochloride salt was purchased from Fluka. All the thiols: 1-octanethiol ($C_8H_{18}S$), 1-decanethiol ($C_{10}H_{22}S$), 1-dodecanethiol ($C_{12}H_{26}S$), octa-decanethiol ($C_{18}H_{38}S$) as well as diethyl ether were purchased from Aldrich. Absolute ethanol denatured with tetrahydrofuran, 1-tetra-decanethiol ($C_{14}H_{30}S$) and 1-hexadecanethiol ($C_{16}H_{34}S$) were purchased from Fluka. Solvents such as methanol, N-methyl pyrrolidone (NMP), dimethyl- formamide (DMF), dimethylacetamide (DMAC), tetrahydrofurane (THF), 1-4-dioxane, and acetone were purchased from Aldrich. Polystyrene sodium sulfonate was purchased from Aldrich was used to prepare a 10mg/mL aqueous solution, the titrant. The following reagents were also all purchased from Aldrich. Gramicidin A of 90% grade; 1,2-dimyristoyl-*sn*-glycero-phosphocholine (DMPC) grade 99%; 2,2,2,-trifluoroethanol grade 99+ %; aniline 99.5% ACS grade, 1-octadecanethiol grade 98.5%. Chloroform spectral grade, lot # B0507212W was purchased from Acros.

Chemical Polymerization of Aniline

Emeraldine salt was synthesized by reacting 50 mL of 0.20M aniline hydrochloride with 50 mL of 0.25M ammonium peroxydisulfate in aqueous medium. Each solution was set at room temperature for 1 hr then mixed, stirred briefly in an appropriate container and left at rest for about 24 hrs to allow polymerization. The polymerized material was collected on a filter, washed with three portions of 100 mL 0.2M HCl then similarly with acetone. The dark green salt PANI was then dried in a vacuum oven at 60 °C.

Preparation of Emeraldine Base

Since the nucleophilic attack of the thiols can be carried out on the base form, PANI salt was then de-doped by stirring it in 0.5M NH_4OH for 8 hrs which change the color from dark green to dark blue instantaneously. This dark blue material is dried the under vacuum for 24 hrs. It can be used immediately or stored in a freezer or in a desiccator protected against light.

Electrochemical Polymerization of Aniline

The syntheses of all polyaniline films were carried out in a three electrodes electrochemical cell filled with 0.1M of distilled aniline dissolved in 0.5M H_2SO_4. The process was controlled by an EG&G Princeton Applied Research, model 263A potentiostat coupled with a PC running the M270 software. All films were grown at room temperature on a platinum plate (50 μm thickness) with a platinum disk as auxiliary electrode polished prior to use with an ultra thin sand paper and an aqueous reference Ag/AgCl reference electrode over a period of 25 minutes. The potential range applied is from -0.15 to $+0.85$ for 50 cycles at a scan rate of 100 mV/s at room temperature. The peak current increased gradually with successive potential scans, which indicates the buildup of the polyaniline film on the electrode surface. Upon completion of the reaction, the film was rinsed in H_2O to remove the anilinium hydrogen sulfate residue and dried at room temperature before measuring the cyclic voltammetry (CV) in 0.5 M H_2SO_4. The film thickness grown at the above conditions was determined by electron microscopy to be about 10 μm on each side of the platinum plate, Figure 4 illustrates the measurement scale by SEM of the typical PANI film.

Reaction of Emeraldine Base (EB) with Alkylthiols

The reaction of EB powder (about 0.5 g) with various 0.1 M alkylthiols in 1:3 ratio of absolute ethanol/chloroform, was carried out under nitrogen at room temperature.[1–3] The reaction of EB with thiols chains ($C_8H_{17}SH$, $C_{10}H_{21}SH$, $C_{12}H_{25}SH$, $C_{14}H_{29}SH$, $C_{16}H_{33}SH$, $C_{18}H_{37}SH$), was carried out for ninety six hours. Simultaneously, the same process was carried out for the short chains thiols (C_8 - C_{14}) over 24 hours period. The purpose of running this second set was to determine the approximate time needed for the reaction. Upon completion of the time scheduled for each set of samples, the resulting products were washed thoroughly with diethyl ether then with acetone and placed in the oven for drying at 60 °C for 24 hrs. The dried product in each case, was ground to powder and characterized by UV-Vis and ATRIR spectroscopy.

Surfase Coating of Polymer Films with Alkylthiols

The films coated with each thiol were consistent in dimensions: 2 × 7 mm and they were all treated the same manner prior to thiol coating: thoroughly rinsed with H_2O followed by methanol then, immersed in 0.1 M anhydrous methanol solution of 1-octanethiol for one day. Upon completion, the film was sequentially washed with methanol to remove the thiols residue, 5% (wt) of aqueous carbonate sodium for 2 minutes to remove the dopant residue (hydrogen sulfate), deionized water to remove the carbonate and acetone to remove the water. PANI film was treated with 1-octanethiol for 1 hour, 1 day and 2 days and the CV was obtained for the coated films before and after soaking in methanol. The same procedure was repeated with 1-decanethiol and 1-dodecanethiol and 1-tetradecanethiol for one day period. For solubility issue, longer chains alkylthiols such as 1-hexadecanethiol and 1-octadecanethiol were prepared in a mixture of 1:10 absolute ethanol and chloroform.

IR Analysis

All experimental measurements were carried out at room temperature. Infrared spectra were recorded using Nicolet ATRIR. The spectral resolution was $4\,cm^{-1}$, scanning rate was $10.0\,cm^{-1}$/sec and number of scanning for sample was 32.

UV-Vis Analysis

Approximately 5 mg of each one of the above powder including Emeraldine Base weighed and dissolved by sonication in 5 mL of the following solvents: THF, 1,4-dioxane, DMAC, DMF and NMP. Subsequently, 50 μL of each sample stock solution was diluted to 3 mL of the same solvent. All samples diluted in DMAC, DMF and NMP were more toward the blue color of EB while those prepared in 1,4-dioxane and tetrahydrofuran were very light in color. The resulting EB samples that have reacted with thiols for 24 hours were dissolved and diluted in DMAC only. UV-Vis spectrum was recorded for each sample in a 1 cm quartz cell from 270 nm to 800 nm using the above mentioned Shimadzu UV-Vis Scanning spectrophotometer.

Cyclic Voltametry

Polyaniline films were deposited using a 0.5 M H_2SO_4 aqueous solution containing 0.05 M aniline monomer using model 660D workstation (CH Instruments, Inc., TX). The electropolymerization of aniline was carried out potentiodynamically on a gold electrode within the potential range of −0.15 and +0.85 V by applying 50 electrode potential cycles at a sweep rate of 100 mV/s. All experiments were performed under nitrogen atmosphere at ambient temperature (25 ± 1 °C).

Potentiometry

For the potentiometric experiments, a two-electrode configuration was setup in which 7mm of a platinum plate coated with PANI or PANI derivatized with thiols was the sample. This system is controlled by a digital voltmeter capable of measuring the potential changed taking place in the solution. The platinum coated EB was immersed in the 15 ml of stirred deionized water until the potential settled. The stability in the potential reading is an indication of equilibrium between the solid material and the water solution. At this point, gradual injection of small volume of the analyte is being made. After each injection, the solution is stirred for 3 minutes and allowed to rest for another three minutes before taking the reading. When there is no significant change in the potential reading, meaning saturation is attained, then, the titration can be considered complete.

Bilayer Assembly

A polyaniline film electropolymerized on a platinum sheet of 50 micron thickness was first coated with 1-octadecanethiol by dipping it in the thiol solution for 72 hours at room temperature. The film thickness on each side of the platinum is about 10 micron. Its overall dimensions are approximately 7 mm long and 2 mm wide with a total thickness of 70 microns.

A computer controlled Langmuir mini-through of 75 mm long and an area of 24300 mm^2, model KSV 2000 series, equipped with a Wilhelmy balance of 39.24 mm of perimeter was used to measure the surface pressure of the area-isotherm, transfer the monolayers of the DMPC and mixture of DMPC/gramicidin. Our experiment requires that three PANI films modified with 1-octadecanethiol to be treated in thiol solution: the first one without any further modification, is titrated with 1 M KCl solution (substrated #1), the second one is to be coated with three layers of only DMPC before titration with KCl (substrate control #2), and the third one is coated at first with 2 layers of DMPC and a third layer forming the artificial membrane with the gramicidin incorporated then titrated with 1M KCl solution. The maximum potential reading reached for the PANI film coated with thiol (substrate control #1) is approximately 20 mV with an initial reading close to zero. In this procedure, the film is first immersed in a specific volume of deionized water stirred for one hour and left to rest until steady potential reading is recorded. At this stage, equilibration occurred then titration starts with gradual pipeting of a 10 mg/mL stock solution of KCl until no change is observed in the potential readings.

For all dipping and the initial isotherm, the substrate used is plain deionized water, no salt added. The mini-through of KSV instrument is filled with approximately two hundred mL (200 mL) of water. For both isotherm determination and formation of DMPC monolayer, temperature control is not required. Prior to each experiment, the KSV instrument is properly cleaned and filled with water. A clean surface was established after hanging the platinum Wilhelmy plate, both barriers and the dipping arm at zero position before starting each experiment. It is important to determine the collapse point of the phospholipid (DMPC) under the investigated conditions to avoid coating the substrate at the solid phase. With a micro syringe, an aliquot of 35 μL of a 1mg/mL DMPC solution prepared in ethanol was carefully spread onto the subphase. The compression starts twenty minutes later, enough time allowed for the solvent to evaporate leaving the DMPC molecules in gaseous state. The film was continuously compressed with the movable Teflon barrier at a compression rate of 5 mm/min and the surface pressure measured with the Wilhelmy platinum plate and the electrobalance. An isotherm shows a change of the slope at a surface pressure below 5mN/m and at an area of 120 cm^2 per molecule of lipid. The monolayer collapses at a surface pressure of 29mN/m, indicating an area of 40 cm^2 per molecule. With this value determined, the target pressure for all subsequent dipping experiment in pure DMPC monolayer should be set at a surface pressure between 23 mN/m to 25 mN/m. However, for the deposition of the gramicidin, since the DMPC solution will be mixed with gramicidin, a true target pressure value cannot be predetermined.

The same 1 mg/mL DMPC solution prepared above is used in the dipping experiment. After cleaning the mini-through and the barriers, water is transferred to it to the appropriate level. The same volume of phospholipids spread onto the subphase and the dipping experiment started after 20 minutes. The isotherm is recorded as part of the dipping process of the substrate in DMPC monolayer. First layer was deposited by dipping the substrate, the second was deposited by pulling

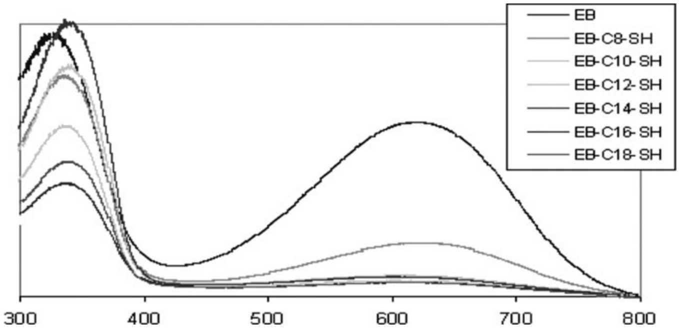


Figure 1.
UV Spectra of PANI after 96 hrs exposure with different alkylthiols, UV medium: DMF.

it from under the subphase and the third one was again carried out by dipping the substrate into the same subphase 20 min later from the second deposition. This time period is necessary to allow the film to dry otherwise, the second layer will most likely be stripped off from the substrate.

In this stage, the final deposition LB film made of mixture of DMPC/Gramicidin is carried out on a thiolated PANI film previously coated with two layers of DMPC. A clean surface is established in the mini-through before spreading the mixture of gramicidin/DMPC solution followed by the deposition of the mixed lipid/peptide film onto the substrate at a target pressure set to 30 mN/m. The transfer ratio (Tr) for this experiment is 2.626 much greater than 1 which is the optimal value for Tr. The subphase was held at 26 ± 2 °C using a digitally controllable waterbath circulator. This temperature controlled is critical to maintain the mixture DMPC/gramicidin above the gel phase transition of DMPC which is about 23 °C.

Results and Discussion

First the optimal conditions for formation of an alkylated layer on PANI using thiolated alkanes were investigated. The carbon segment length in the alkylthiols varied from C8 to C18 and five different solvents were used to monitor the optimal nucleophilic substitution reaction. UV-Vis spectroscopy was used for the initial analysis of the degree of thiolation. The typical UV-Vis spectra of EB solution is characterized by two major absorption bands (Figure 1), one at about 320 nm and 630 nm. The first band is associated with π-π^* transition of the conjugated rings systems ($\sim$4 eV), associated with the delocalization of the benzene π-electron to the nitrogen of the amine, and the second (2 eV) is attributed to the excitation from the highest occupied molecular orbital (HOMO) of the benzenoid rings to the lowest unoccupied molecular orbital (LUMO) of the quinoid rings. This is an indication that conjugation does occur along the polymer chain between the aromatic rings and the p-orbital of the nitrogen lone pair electrons. As observed in Figure 1, the intensity of the band at 630 nm associated with the quinoid ring (Q) has decreased in intensity for all EB-thiols products compared to plain EB as the band at 320 nm associated with the benzenoid ring (B) remains unchanged. This decrease in intensity indicates that the reaction occurred with the conversion of the strained quinoid peak to a more stable benzenoid peak.

The ratio of the 630 nm and 320 nm intensities relate the quinoid and benzenoid units with the thiolation reaction (Q/B ratio) and thus the decrease in this ratio measures the reaction semi-quantitatively. We present the use of all the alkylthiols in the five different solvents in Figure 2. In the

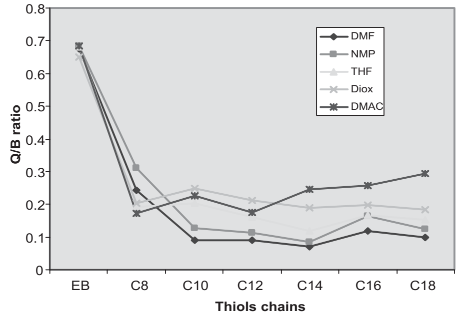


Figure 2.
Profile of Q/B ratios of all EB-thiol products in different solvents.

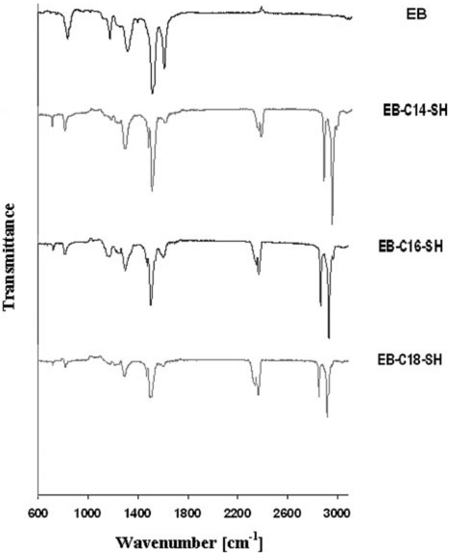


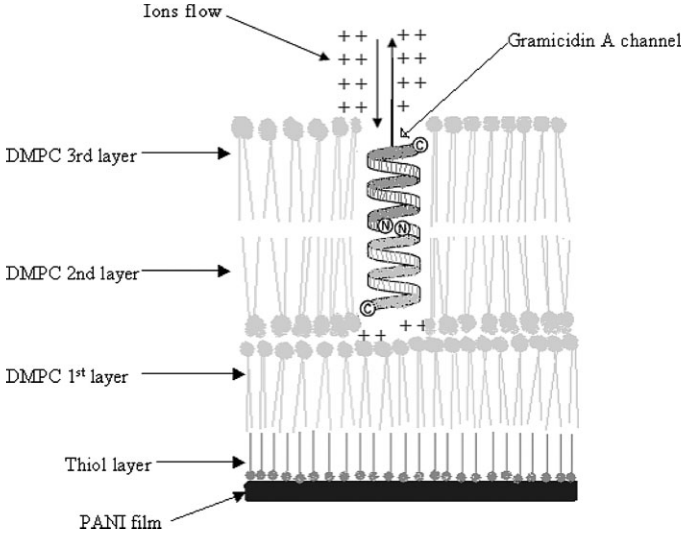
Figure 3.
Peak identification from overlay of IR spectra for EB and derivatized EB with all thiols.

solvents studied, emeraldine base exhibits a decrease of the Q/B ratio with all alkylthiol but the largest change is with octadecylthiol in DMF. We also observed in additional experiments that 96 hours of thiolation reaction was needed as compared to a 24 hr experiment.

IR spectroscopy was similarly used for the evaluation of the thiolation reaction. The spectra exhibit similar features and the differences observed between them can be attributed to the thiols chains length attached to the rings. The peaks at 1600 cm^{-1} and 1500 cm^{-1} are related to the carbon-carbon stretching vibrations in the quinoid and benzenoid rings respectively (see Figure 3). Han et al reported the same intensity reduction observed in the overlay spectra for peak at 1600 cm^{-1} which is attributed to a C=C stretching vibration of the diiminoquinoid ring and peak at 1170 cm^{-1} (a vibration mode associated with diiminoquinoid ring. A very weak absorption band is observed at 720 cm^{-1} due to methylene rocking. According to Han et al,[2–4] peak at 820 cm^{-1} a C–H is out of plane vibration, peak at 1169 cm^{-1} and peak at 1600 cm^{-1} seem to all decrease in intensity similarly to reduction observed when EB is reduced by hydrazine to leucoemeraldine. The bands at 3000–2800 cm^{-1} should be expected to increase in intensity with the percentage of alkylation. For thiols with chain lengths of 14 to 18 carbon atoms the intensities of these bands increase significantly. Again the comparison of peaks related to Q and B reveals the effective thiolation as the peak at 1600 cm^{-1} disappears almost completely. This interpretation is consistent with the observation in the IR spectra of EB derivatized with thiols. The very weak band observed at 3400 cm^{-1} is indicative of N–H stretch.

The drastic decrease of the Q/B ratio extracted from the UV-Vis data for EB-thiols products versus plain EB in different solvent indicates clearly that the alkylthiols chains did substitute PANI in all cases. The variation observed in the Q/B ratio from among thiol chain length was a combination of solvent effect on the quinoid rings and possible side products generated in the reaction medium. The optimal degree of thiolation occurred with1-octadecanethiol in DMF and this modification was selected for the further steps in the electrode modification.

Scheme 1 illustrates the architecture of the thiolated PANI supported bilayer system with gramicidin A channel embedded. PANI electrode was first thiolated with 1-octadecanethiol dipped into a DMPC solution and eventually into a



Scheme 1.
Schematic representation of Gramicidin A channel incorporated in DMPC bilayer supported on a previously thiolated PANI film.

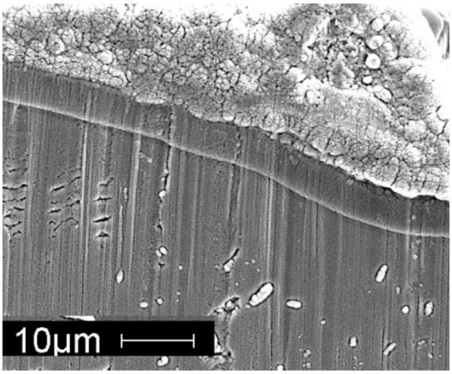


Figure 4.
SEM micrograph of a cross-sectional area of typical PANI film on Pt plate after 50 electrode potential cycles at a scan rate of 100 mV/s. Magnification shown in the Figure.

DMPC solution with gramicidin A. The theoretical thickness of this system including the platinum surface, is roughly estimated to 68.61 nanometers considering that DMPC molecule length is about 21.7 ± 0.5 Å at 20 °C and octadecylthiol being estimated at 21 Å. The shift of the isotherm upon addition of DMPC/gramicidin A to the subphase (75 mN/m) in comparison to pure DMPC (55 mN/m) monolayer indicates that the gramicidin A is incorporated. The macroscopic image of the PANI layer is shown in Figure 4.

The electrochemical analysis of the films is presented in Figure 5. A is the CV prior modification with thiol, B is the same film after thiol modification for two days, and C is the film after three days of thiolation. In film A, the typical two redox couples are observed for PANI film. The first couple at a potential of 0.08 V corresponds to emeraldine/leucoemeraldine transition and the second at a potential of 0.67 V corresponds to pernigraniline/emeraldine transition. In some cases, a third redox couple is depicted in between the above two. Such redox couple is reported to be due to other chemical reactions during polymerization. After modification with thiol, the two redox couples start to move closer to each other. Eventually, the peak intensities also decrease showing that alkylthiol renders PANI in an electroinactive state similarly as has been observed when based in buffer (PBS, pH 7.3).

On the other hand, Figure 6 illustrates that the potentiometric response of gramicidin incorporated in DMPC bilayer with a maximum reading of 86 mV. In this proce-

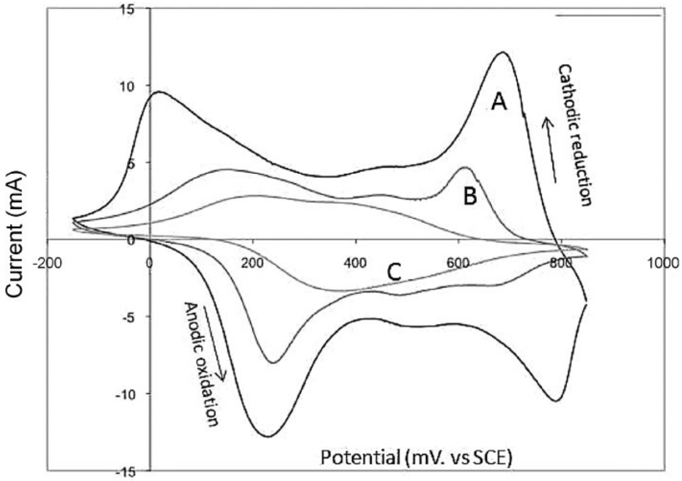


Figure 5.
CV of Platinum coated PANI: (A) CV before reaction with thiol, (B) CV after 2 days (C) CV after 3 days treatment with 0.1 M of 1-Octadecanethiol in 1:10 ratio (absolute methanol/chloroform). Anodic oxidation with negative current.

dure, the film was rather immersed sequentially in a series of KCl solutions for which the concentration range was between 0 mg/mL to 0. 05 mg/mL. The film titration of the film with 1 M KCl using a digital voltmeter reached potentials around 20 mV when the controls of thiolated PANI and PANI with 3 DMPC layers were used. The value for the working electrode with the gramicidin ionophore reached over 80 mV values (Figure 6). This value is higher than the maximum obtained for both controls,

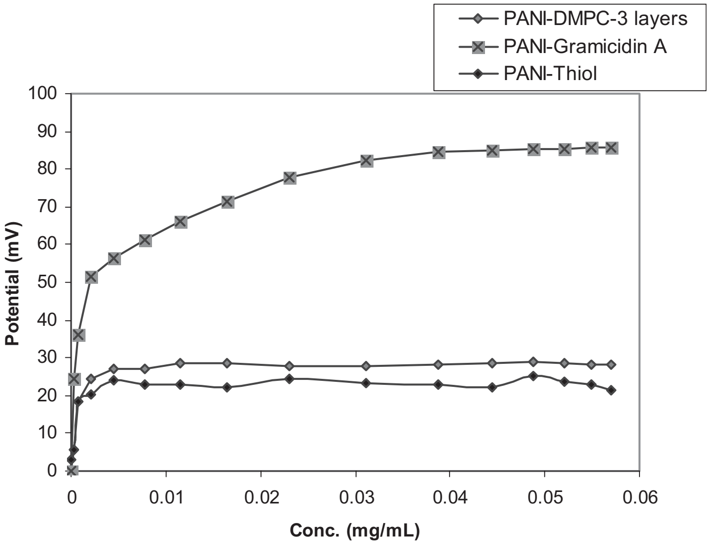


Figure 6.
Potentiometric response of gramicidin to K^+ in DMPC bilayer by dipping method.

the thiolated substrate and the substrate covered with DMPC. The diffusion of K^+ ions is more pronounced than what was observed for the PANI-thiol and PANI-DMPC substrates and confirms that the ionophore gramicidin provides a selective component on the membrane. Based on the potentiometric responses, we can also conclude that the bilayers seal the polyaniline surface well and that gramicidin has been incorporated within the DMPC bilayer.

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