Thromboresistant Chemical Sensors Using Combined Nitric Oxide Release/Ion Sensing Polymeric Films

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Efforts to develop implantable chemical sensors, both optical and electrochemical, capable of monitoring physiologically important ions (H^+, K^+, Na^+, etc.) and gases (CO_2 and O_2) continuously via intra-arterial catheters have met with only limited success owing to the difficulties in fabricating fully functional analytical devices that are nonthrombogenic.[1,2] At the same time, placement of such miniaturized sensors within blood vessels can lead to vasoconstriction of arteries, thereby reducing blood flow which, in turn, can lead to sensor output signals that do not correlate with the true ion and/or gas levels within the bulk blood. Herein we demonstrate that it is now possible to fabricate chemical sensors that emit low levels of nitric oxide (NO), a potent platelet antiaggregation[3] and vasodilating agent,[4] over extended periods (days) without impairing the analytical performance of the sensing devices, and that the surfaces of the resulting sensors show a marked decrease in thrombogenic properties, as measured by *in vitro* platelet adhesion studies.

As model chemical sensors, we prepared classical polymer membrane type ion-selective electrodes (ISE) for H^+ and K^+ by doping Tecoflex polyurethane (PU) (Thermedics Inc.) or conventional high-MW poly(vinyl chloride) (PVC) films (150 μm thick) with the highly selective ionophores tridodecylamine (TDDA)[5] and valinomycin (VAL),[6] respectively, along with appropriate levels of a lipophilic anionic site additive, potassium tetrakis(4-chlorophenyl)borate (KTpClPB);[7] all membranes were plasticized with dioctyl sebacate (DOS). To adapt these polymer films for both electrochemical ion sensing and simultaneous NO release, the membranes were prepared as multilayer films in which three layers of equal thickness were cast sequentially. The solution for each layer was obtained by dividing a conventional 3.0 mL ISE membrane cocktail with varying wt % of polymer and plasticizer into thirds (the wt % of TDDA and VAL was 2.5%, and the wt % of KTpClPB was 0.6−1.7%, in all cases). The first and third layers were cast without modification, while the second (middle) layer was cast after the addition of 10.0 mg (4 wt %) of *N,N′*-dimethylhexanediamine nitric oxide adduct (DMHD/N_2O_2), a diazeniumdiolate species that has already been shown to generate NO spontaneously in aqueous solutions, with a solution half-life of 1.0 min at 37 °C.[8,9] Although this compound is not soluble within the organic phase, a fine dispersion was obtained by sonication.

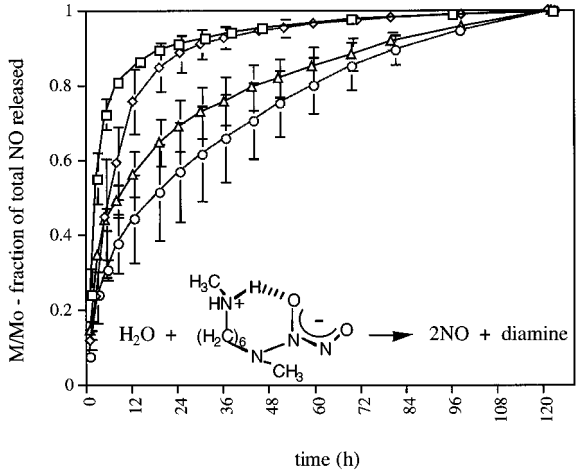

Figure 1. Nitric oxide release curves for H^+-selective multilayer films of the following compositions by wt %: (□) 66% PU/30% DOS/1.1% KTpClPB/2.5% TDDA; (◇) 66% PVC/30% DOS/1.1% KTpClPB/2.5% TDDA; (○) 33% PU/63% DOS/1.0% KTpClPB/2.5% TDDA; (△) 33% PVC/63% DOS/1.1% KTpClPB/2.5% TDDA. Each curve represents the average of three trials with representative standard deviations observed over the measurement period.

Before evaluating the analytical response properties and potential blood compatibility of the polymeric films, the release of NO from the multilayer membranes containing DMHD/N_2O_2 was assessed. Confirmation that gaseous NO is, in fact, released was made using a hemoglobin-based spectrophotometric assay in which oxyhemoglobin, Hb(Fe^{2+})O_2, is rapidly converted to methemoglobin, Hb(Fe^{3+}), via reaction with NO.[10,11] More quantitative data for the release of NO from the multilayer, ion-selective polymeric films was achieved by measuring the amount of nitrite ions generated in the bathing solution over extended soaking periods. Because NO reacts almost immediately (half-life of several minutes compared to the time frame of the experiment (days)) with water and oxygen to yield nitrite ($4NO + O_2 + 2H_2O \rightarrow 4NO_2^- + 4H^+$),[12] the accumulated nitrite concentration in the bathing solution is directly proportional to the total amount of NO released from the NO adduct. Figure 1 shows the rate of NO release, measured indirectly as nitrite using a flow-injection analysis system equipped with biamperometric detection,[13] for various H^+-selective PU and PVC films doped with DMHD/N_2O_2. Significant variation in the rate of nitric oxide release is obtained by altering the polymer/plasticizer composition; indeed, a substantial decrease in release rate is observed for all films composed of a 1:2 polymer/plasticizer ratio compared to those containing a 2:1 polymer/plasticizer ratio. This observation may be explained by the model proposed by Li *et al.* in which more elastic membranes allow larger immobile water droplets to form within the film, but such water diffuses much more slowly within the organic phase.[14] This, in turn, decreases the rate at which the NO adduct encounters water molecules, resulting in a slower NO release rate. Similar results have been obtained for K^+-selective membranes, and in all cases, the rate of NO release from the DMHD/N_2O_2-doped films is far slower than predicted on the basis of the NO adduct's half-life (1 min) in aqueous solution.

To demonstrate that the films which emit NO also remain permselective for H^+ and K^+, the potentiometric ion response

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Table 1. Potentiometric Properties of Blank and DMHD/N_2O_2-Doped Membranes

membrane composition	slope[a] (mV/decade)		log k^{pot}_{i,Na^+} [b]	
	blank	doped	blank	doped
66% PU/30% DOS/0.6% KTpClPB/2.5% VAL	60.6 ± 0.2	60.3 ± 0.1	−5.44	−5.31
33% PU/63% DOS/1.7% KTpClPB/2.5% TDDA	59.7 ± 0.6	58.5 ± 0.9	−9.50	−9.38
66% PU/30% DOS/1.7% KTpClPB/2.5% TDDA	56.5 ± 1.8	52.0 ± 2.9	−9.35	−9.31

[a] Data are mean ± SD for a minimum of three electrodes. [b] By fixed interference method.[29]

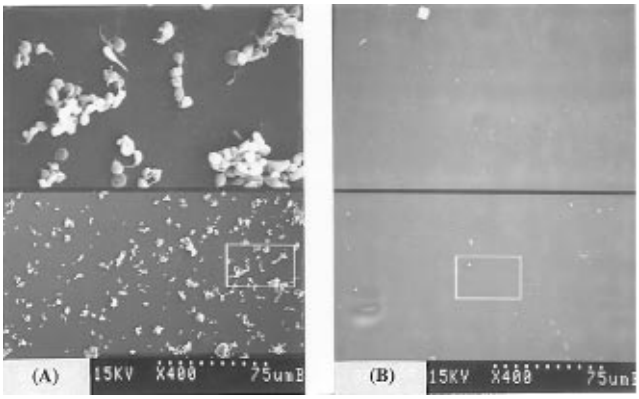

Figure 2. Scanning electron micrographs of 66% PU/30% DOS pH sensing membranes with (B) and without (A) DMHD/N_2O_2 following incubation in platelet-rich sheep plasma. The top portion of each micrograph represents a 5-fold increase in magnification of the area selected in the bottom portion.

properties of the membranes were investigated and the results obtained are presented in Table 1. As shown, only a slight decrease in slope is observed for doped membranes compared to conventional (blank) ISE films formulated without the NO adduct; in addition, the selectivity coefficients for H^+ over Na^+ (in the case of TDDA membranes) and K^+ over Na^+ (in the case of VAL membranes) remain essentially unchanged for membranes containing DMHD/N_2O_2 compared to blank films. These potentiometric response properties demonstrate that the presence of DMHD/N_2O_2 and the concomitant generation of NO does not interfere with the selective interfacial chemistry required to generate analytically useful potentiometric signals from the doped ion-sensing films.

While ISE polymeric membranes prepared with and without the NO-generating compound display comparable analytical performance with respect to ion sensing, the resulting multilayer films behave quite differently with regard to their potential thrombogenicity. An established procedure using platelet-rich sheep plasma[15−17] was used to assess platelet adhesion to the films *in vitro*, since such tests are considered by many researchers to be informative as an initial measure of thromboresistivity in humans.[18−20] As shown in Figure 2A, the scanning electron micrograph of an undoped PU-based pH sensing membrane after exposure to platelet-rich sheep plasma shows a high number of activated platelets adhered to the membrane. In contrast, ion-sensing films that contain the DMHD/N_2O_2 compound (Figure 2B) consistently have relatively few platelets adhered, and those that are present are not activated, as indicated by the lack of pseudopod extensions. Such results have been observed repeatedly and are in agreement with *in vitro* as well as *in vivo* data recently reported by Smith et al. in which various diazeniumdiolate compounds have been incorporated into polymers to enhance the biocompatibility of these materials.[21] However, in the work reported here, the polymer films not only release NO but also serve simultaneously as analytical transduction elements for selective ion sensing.

The approach described herein should be applicable for enhancing the biocompatibility of previously reported polymer tubing-based potentiometric pH, CO_2, and K^+ sensing catheters[22,23] that have been shown to function effectively *in vivo*, but only when test animals are systemically anticoagulated. It is also likely that the use of diazeniumdiolates or related species to emit NO will be compatible with the development of implantable amperometric chemical sensors (e.g., oxygen sensors)[24] as well as optical ion/gas sensing catheters.[25] With the use of sensors that continuously emit NO, nondesired systemic anticoagulation may not be required. While potential toxicity concerns will need to be thoroughly investigated, the amount of NO released per minute per unit area of the polymer films reported here is far less than that endogenously released by porcine aorta endothelial cells[11,26] (the porcine cardiovascular system is similar to that of humans[27]). Indeed, the amount of NO released from an implanted catheter (0.63 cm^2) made from such films over the course of 24 h would be far less (<0.3%) than the 1 mmol or more of NO produced endogenously each day in humans.[28] Nonetheless, more detailed studies will be required to determine the optimal polymer film compositions which will generate the surface levels of NO required to achieve the greatest decrease in platelet adhesion and activation over extended time periods. Further, once the sensors are tested *in vivo* via catheter-type devices, it will be possible to determine conclusively whether the continued release of NO from these analytical devices effectively dilates the local arteries in which the sensors are placed, thus maintaining adequate blood flow for accurate measurements of critical blood gas and electrolyte species. Such experiments are currently in progress in collaboration with scientists in the Department of Anesthesiology at The University of Michigan.

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Supporting Information Available: Experimental procedures for confirming the membrane release of NO using the spectrophotometric assay and the *in vitro* platelet adhesion studies (2 pages). See any current masthead page for ordering and Internet access instructions.

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