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ARTICLE *in* ANALYTICAL CHEMISTRY · DECEMBER 2001

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Fiber-Optic Luminescent Sensors with Composite Oxygen-Sensitive Layers and Anti-Biofouling Coatings

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Anti-biofouling polymers containing phosphorylcholine (PC)-substituted methacrylate units have been prepared by copolymerization with dodecyl methacrylate and used to coat luminescent oxygen sensors. Nanometer-sized coatings of such materials are shown to reduce significantly the adhesion of marine bacteria (more than 70%) and thrombocytes (more than 90%) to the surface of tris(4,7-diphenyl-1,10-phenanthroline)ruthenium(II)-doped silicone layers. A thorough analytical characterization of both the PC-coated and the uncoated dyed films has demonstrated that the anti-biofouling layers do not alter dramatically the performance of the fiber-optic oxygen sensors in aqueous media and are mechanically stable for more than one year of continuous immersion. The slope of the linear calibration plots in the 0−8 mg L^{-1} oxygen concentration range (ca. 1.0 L mg^{-1}) decreases 8−11% after applying the 50-nm protective layer with no change in the sensor precision (1.1−1.9% RSD, $n = 6$). The response time of the 200-μm O_2-sensitive layers (1.5−6 min) increases up to 2-fold, depending on the nature of the PC polymer used, but the temperature effect on the sensor response (0.020 L mg^{-1} $°C^{-1}$) remains essentially unchanged. Oxygen detection limits as low as 0.04 mg L^{-1} have been measured with the coated optodes. The novel biofouling-resistant optosensors have been successfully validated against a commercial oxygen electrode and are shown to respond faster than the electrochemical device for large oxygen concentration changes. The biomimetic coatings will be particularly useful for drift-free long-term operation of environmental optosensors and in vivo fiber-optic oxygen analyzers.

Continuous monitoring of medical parameters, biological species, and industrial processes is becoming increasingly critical worldwide. At the same time, more stringent regulations call for continuous in situ environmental monitoring of water quality parameters by means of deployed sensors. One of the most significant problems when using sensing devices in the real world is surface fouling, that is, the rapid accumulation of adsorbed material on the working surface of sensors. Such a foreign material leads to drift and eventually sensor failure as a result of disruption of the sensitive material or prevention of analyte transport into the sensitive reagent layer. A major cause of concern, particularly in environmental, clinical, or bioreactor monitoring, is the formation of protein layers or the adhesion of microorganisms and cells.[1−3] Sensor biofouling makes long-term monitoring difficult and requires frequent (field) maintenance operations and probe replacement. Moreover, intravascular sensor systems and artificial bedside monitoring devices used for extracorporeal circulation that come into contact with blood demand smart materials that are able to prevent thrombus formation.[4,5]

Sensor biofouling is common to every monitoring technique that operates in situ (electrochemical, optical, electrical, thermal, etc.). Among the strategies currently employed to prevent or minimize membrane biofouling of implanted (bio)sensors, the following are being applied:[1,6] novel polymer materials and coatings (latex-based, biodegradable, nitrogen monoxide-releasing, tyrosine derivatives, star polymers, hydrogel overlays, stronger chitin, Nafion, etc.); anticoagulant heparin coatings; phospholipid-based biomimicry; physicochemical surface modification (irradiation, plasma, corona, texturing, ozonization, silanization, photocoupling, etc.); surfactant-modified, natural and drug-derived membranes; diamond-like carbon coatings; and flow-based systems combined with the in vivo sensors. From the environmental point of view, anti-fouling protection has been accomplished by simple agitation of the sensor, polymer membranes containing biocides,[7] piezoelectric actuators,[8] pulsed electric fields,[9] Cl_2 or

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10.1021/ac015517n CCC: $20.00
Published on Web 09/19/2001

biocide-release mechanisms,[10] enzyme coatings,[11] biodispersant treatments, and ozonization systems.[12]

Thanks to popularization of fiber-optics, spectroscopy has jumped the gap that separates laboratory from field measurements and is becoming a viable alternative to conventional sensors in which miniaturization, an absence of electromagnetic interferences or electrical risks, and ruggedness, is a must.[13] Fiber-optic oxygen sensors based on luminescence measurements have been the subjects of active research in the past 20 years.[14,15] More than a dozen patents have been issued that describe indicators, devices, and procedures to quantify such analytes in the liquid or gas phase using excited-state quenching of an appropriate indicator by molecular oxygen. Some dedicated instruments have already been commercialized to monitor oxygen in biomedical or environmental areas;[16] however, no studies on biofouling of the sensitive membranes have been reported so far as a result of the fact of probe disposability or the absence of extended in situ testing of the current sensors. Actually, substantial clotting around the fiber-optic catheter has hindered the development of a commercial oxygen optode to monitor this gas in the blood vessels of patients in intensive care units.[17,18] In general, the same situation exists if luminescent sensors are to be used for long-term water monitoring. Bacterial and microorganism adhesion on their working surfaces preclude the use of many otherwise excellent sensors. Therefore, thrombocytes or marine bacteria may well constitute two representative challenges to test the protective features of anti-biofouling layers.

Phosphorylcholine (PC) is the major lipid headgroup component found in the outer surface of biological cell membranes. PC-containing polymers, when coated onto the surface of a number of medical devices, have been shown to reduce interactions with body fluids, such as blood, tear film, and urine.[19] The PC group is polar, having both negative and positive charges, but is overall electrically neutral. It has a great affinity for water and is able to minimize irreversible protein binding to surfaces. Biocompatibles Plc. has utilized the methacrylate monomer (**1**, Figure 1) to make a range of polymers with differing physical and chemical properties. For example, solution copolymerization of **1** with dodecyl methacrylate, hydroxypropyl methacrylate and trimethoxysilylpropyl methacrylate yields a film-forming cross-linkable polymer system with both hydrophilic and hydrophobic domains that is capable of adhering to many materials. It is used on the BiodivYsio coronary stent as a means of rendering the device biocompatible.[20]

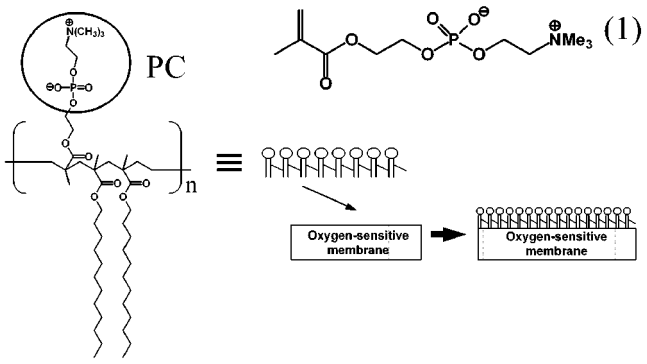

Figure 1. Chemical structure of the methacrylate monomer containing a phosphorylcholine (PC) moiety (**1**) and chemical backbone of the PC1036 and PC1008 polymers used as protective layers in this study.

The aim of our study has been to demonstrate the feasibility of using biocompatible anti-fouling coatings on optical sensing materials. These coatings might be used both in direct (through-window) spectroscopic measurements and in luminescent fiber-optic sensors that are based on indicator chemistries. To that end, two PC-containing polymer materials were developed for anti-biofouling protection of silicone films loaded with an oxygen-sensitive luminescent dye. The analytical features of the PC-coated vs the uncoated sensors have been thoroughly investigated in order to check the effect of the protective layers on the dissolved oxygen measurements. In parallel with more fundamental studies to characterize the novel PC surfaces from a physicochemical and topographical point of view,[21] a kinetic study of bioadhesion phenomena under the influence of simulated marine surroundings, as well as in platelet-enriched plasma, has been undertaken, too.

EXPERIMENTAL SECTION

Fabrication of Indicator Membranes. The oxygen-sensitive luminescent membranes are made from a commercial poly(dimethylsiloxane)-based formulation (Dow-Corning 3140, Wiesbaden, Germany) and 10% (by weight) fumed silica gel as additional filler (S5130, Sigma-Aldrich Quimica, Madrid, Spain; >99.8% SiO_2; bead size, 7 nm; surface area, 390 $m^2\ g^{-1}$; density, 3.68×10^{-2} g cm^{-3}), which is required to adsorb the ionic indicator dye after curing. To prepare silicone films, the polymer is manually mixed thoroughly with the appropriate amount of filler until a very thick homogeneous mixture is achieved. A thin layer of the mixture is immediately spread onto 75 × 25 mm pieces of 100-μm polyester film with the aid of a homemade constant thickness film maker and a sharp blade. The composite film is allowed to cure for 7 days at room temperature and humidity to yield membranes of (200 ± 20)-μm thickness. Although the films show

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no "tack" after 3 days, we have noticed that the efficiency of the dyeing process is far from optimum, unless they have been left under those conditions for the specified time. The polyester support is detached by immersing the composite into dichloromethane for a few seconds. Then the luminescent indicator, tris(4,7-diphenyl-1,10-phenanthroline)ruthenium(II) dichloride (RD3),[22] is loaded onto the silica gel filler upon introduction of the undyed silicone film into a 0.25 mM solution of the metal complex in dichloromethane for a few minutes, and there is a large amount of resultant swelling produced in the membrane. After rinsing with dichloromethane, the dyed membranes are allowed to air-dry for 3 days in order to allow solvent evaporation and reversion to normal size. At this point, they are ready for coating with a thin PC polymer layer.

To produce an oxygen-sensitive membrane coated on one side only, the following procedure was adopted. The membranes were wiped clean with ethanol, and two of them were placed back to back. The external surfaces were then plasma-pretreated (conditions: air plasma, 3 mL s^{-1}; 100 W, forward power; 5 W, reflected power; 1 min dwell time per side) to make them wettable by the PC polymer coating solution. The membranes were dip-coated within 5 min of pretreatment in a 1% (by weight) PC polymer solution in ethanol (Biocompatibles Ltd., Farnham, U.K.) at a speed of 3 mm s^{-1} and were then peeled apart and allowed to air-dry. The samples coated with PC1036 (a film-forming acrylic copolymer containing 23% PC and silane functional cross-linker)[23] were then cured overnight at 70 °C. Membranes coated with PC1008, an acrylic copolymer containing 33% PC and having hydrophobic film-forming units to produce an adsorbable coating,[23] were not subject to thermal posttreatment. The dip-coating procedure yields a PC polymer layer in the order of 50 nm, as measured by atomic force microscopy.

The coated or uncoated indicator membranes were cut in 3.5-mm diameter disks and immersed for at least 6−7 days in purified water (Millipore Milli-Q system), containing 0.1% sodium azide as preservative before investigating their oxygen response.

Instrumental Setup. The luminescence-quenching measurements were performed at 25.0 ± 0.5 °C and barometric pressure in Madrid (typically 698−713 Torr), under moisture-saturated nitrogen/oxygen mixtures prepared from pure gas cylinders (Carburos Metalicos, Spain) with electronic mass-flow controllers (ICP-CSIC, Madrid, Spain). The gas mixtures (200 mL min^{-1} overall) were delivered via 1/8-in.-o.d. nylon tubing to a thermostated (Polyscience model 911, New York) glass reservoir containing ca. 3 mL of laboratory-purified water. Using a drilled stainless steel screw cap, a 3.5-mm disk of the composite oxygen-sensitive membrane was attached to the stainless steel-terminated common end of a fused-silica bifurcated fiber-optic bundle (Fiberguide Ind., New Jersey; 30 fibers, 2-m overall length, 1.5-mm diameter at the common end, 1-mm diameter at each branch). Each branch was connected to the light source and spectrograph entrance of a homemade portable spectrometer (LFA-1000). The LFA-1000 consisted of a 12 V, 10 Hz pulsed xenon lamp (Ocean Optics Europe, Holland), the emission of which was filtered through a 400-nm-wide band-pass filter (Schott, Germany), and a MS-256 1/8-m spectrograph (600 grooves mm^{-1} grating; Oriel, CT) fitted with a 530 nm cutoff filter (Schott, Germany) in front of a 1.2-mm entrance slit. A thermoelectrically air-cooled (at 5 °C) Instaspect IV CCD detector (1024 × 256 pixel; Oriel, CT) was fixed to the exit port of the spectrograph. Lamp pulsing, detector synchronization and powering, data acquisition, and signal processing were provided by a laptop 486DX100 computer (KWM, Taiwan), attached to a docking station containing the Instaspect IV interface card, using the original Oriel software.

Sensor Validation. The validation of the O_2-sensitive PC-coated membranes was carried out with the help of a calibrated electrochemical sensor (WTW Oxi 340-A, Germany). A double glass chamber containing ca. 6 mL of laboratory-purified water was designed to measure simultaneously the dissolved oxygen level in water using both sensors (Clark-type and optode). The other working conditions (physical and instrumental) were kept as described above. The electrochemical sensor was calibrated each day at the beginning of the oxygen measurements following the manufacturer protocol. The Clark oxymeter was connected to the serial port of a laptop computer (KWM 486DX4) in order to log the oxygen concentration values every 5 s.

Bioadhesion Studies. Microorganism adhesion to the PC-coated and uncoated oxygen sensitive membranes was monitored by means of a computer-controlled bioreactor system (Celligen Plus, New Brunswick, Germany), which allows a reproducible and continuous cultivation of different cells or bacterial strains. The bioreactor was connected to a homemade flow-through chamber where the silicone membrane to be tested (75 × 25 × 0.2 mm) is placed (Figure 2). Because of the transparency of the investigated coated and uncoated RTV silicone layers, the flow-through chamber was adapted for direct microscopic observation (Olympus BX 60, Germany). On-line measurements of the cell adhesion kinetics with light microscopy and image processing techniques (Analysis Pro, SIS, Germany) were performed to quantify the number of adhered cells or microorganisms on each material surface (Figure 2) under continuous and reproducible streaming conditions of 2.5 mL min^{-1}. To simulate the marine environment, a mixed culture of marine denitrifying bacteria enriched with *Thiobacillus denitrificans* (7.5 × 10^6 cells mL^{-1}) was employed. Additional biofouling tests were carried out in a thrombocyte suspension consisting of fresh human plasma enriched with 2 × 10^8 cells mL^{-1} in order to simulate the contact of sensors with blood (Institut für Transfusionsmedizin, Suhl, Germany).

RESULTS AND DISCUSSION

Analytical Characterization. Laboratory experiments (see below) have shown that PC-containing acrylic polymers indeed protect luminescent oxygen sensors against adhesion of cells and proteins. However, it remained to be determined how much the anti-biofouling coating affects their sensitivity to the measurand. The analytical characterization and validation of PC-coated oxygen sensing layers containing the luminescent RD3 indicator was carried out in comparison to the uncoated films. The parameters evaluated for the oxygen optodes were their response interval, calibration curves, detection limits, precision, reproducibility, and the stability of the measurements. Under the working conditions described above, typical response functions of the fiber-optic sensors for different oxygen levels in water are shown in Figure 3. In every case, the relative emission intensity (I_0/I) values at 25 °C in the 0−8 mg L^{-1} oxygen concentration range (corresponding

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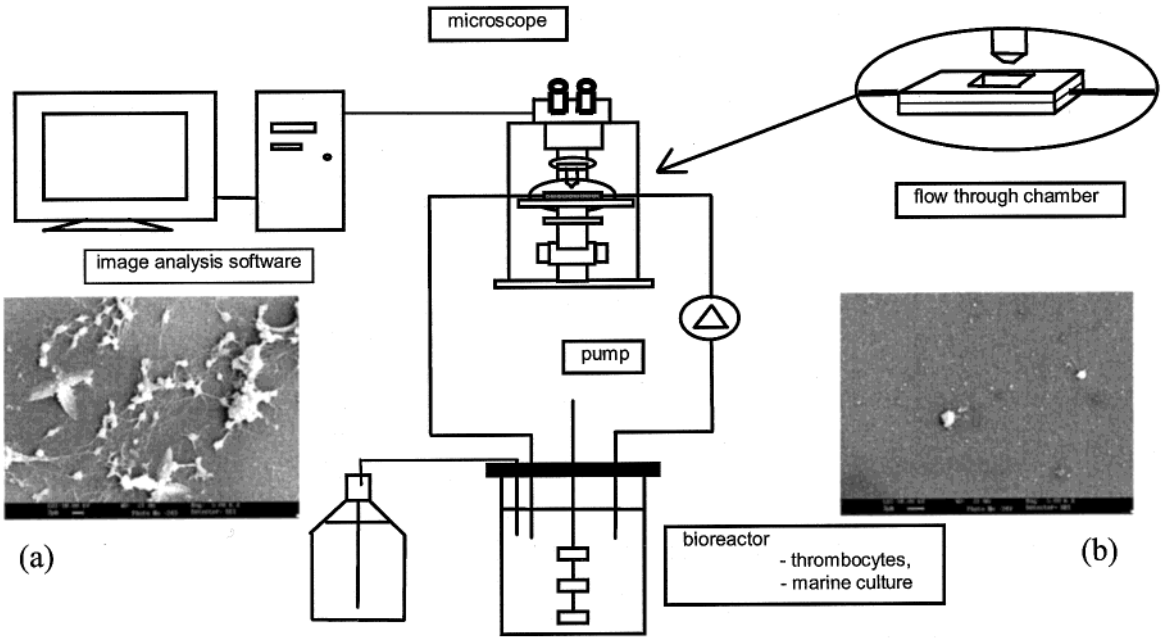

Figure 2. Reactor system for kinetic bioadhesion tests in fouling media of the PC-coated and uncoated oxygen-sensitive silicone films using microscopy and image analysis. Insets: photomicrograph of thrombocytes adhered to (a) uncoated and (b) PC1008-coated Ru-doped silicone films (see text).

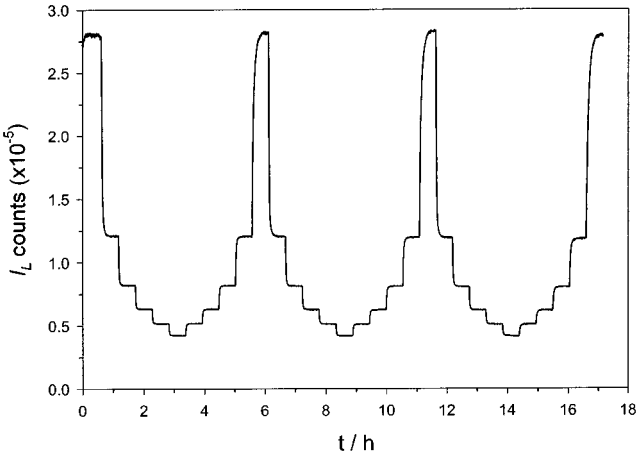

Figure 3. Typical response function of the fiber-optic luminescent sensors to different oxygen concentrations (0, 1.4, 2.8, 4.2, 5.6, and 7.4 mg L^{-1}) in water.

to 0−21% analyte in the gas phase equilibrated with the water samples) can be fitted successfully to a linear regression ($r >$ 0.996) using the classical Stern−Volmer equation. However, at higher oxygen levels, a slight downward curvature was noticed. This behavior is possibly due to some inhomogeneity of the dye binding sites on the silica gel that lead to differences in the local oxygen quenching rate constants.[24] The slope of the calibration plots is a function of the membrane immersion time. Values ca. 1−5% higher than equilibrium are measured at the beginning of the monitoring experiment (i.e., 6−7 days after the initial placement in water), but the oxygen sensitivity stabilizes after ca. 15 days in water. Under these conditions, the indicator layer dose/response plots show slopes of 0.99 ± 0.01, 0.91 ± 0.03, and 0.88 ± 0.04 L mg^{-1} for the uncoated, PC1008- and PC1036-coated membranes, respectively (standard deviation of 60 calibration plots for one sensor disk measured discontinuously over 60 days). At any time and if we also take into account the variability introduced by the sensor fabrication process (see below), the average sensitivity of the uncoated luminescent membranes is 9% higher than the PC1008-coated and 13% higher than the PC1036-coated silicone layers. Probably the PC polymer coating process, which is applied to the fully cured Ru-doped films by dip-coating in organic solvent, makes "foreign" molecules adsorb onto the outermost silica gel particles, where the luminescent dye is also immobilized and where the oxygen quenching takes place.[25,26] Such coadsorption decreases binding of molecular oxygen and, therefore, the efficiency of the excited-state deactivation process for a given analyte level. Moreover, the PC1036 polymer has functionality capable of reacting with hydroxyl groups at the surface of the silica gel. Coating the sensitive membranes with other materials (e.g., black silicone for optical isolation) has also been demonstrated to diminish the oxygen sensitivity of the RD3/silica gel-containing silicone layers.

The decrease of the oxygen sensitivity as a function of the immersion time in water must be attributed to a slow hydration process of the 200-μm silicone membrane filled with dyed silica gel.[27] Initial autoclaving of the sensing membranes would probably avoid the hydration effect,[27] as has been observed in our laboratory for similar indicator membranes (data not shown). The thin PC polymer coating does not seem to affect membrane hydration kinetics. After being immersed for one year in water, no evidence of peeling of the coating from the silicone substrate or degradation of the latter has been found. Therefore, the cross-linkable PC

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Table 1. Precision[a] of the Fiber-Optic Luminescent Sensors to Different Oxygen Concentrations in Water

$[O_2]$, mg L^{-1}	uncoated	PC1008	PC1036
1.4	1.9	1.3	1.8
2.8	1.5	1.4	1.9
4.2	1.8	1.5	1.9
5.6	1.1	1.4	1.8
7.4	1.8	1.5	1.1

[a] Expressed as RSD in %, $n = 6$.

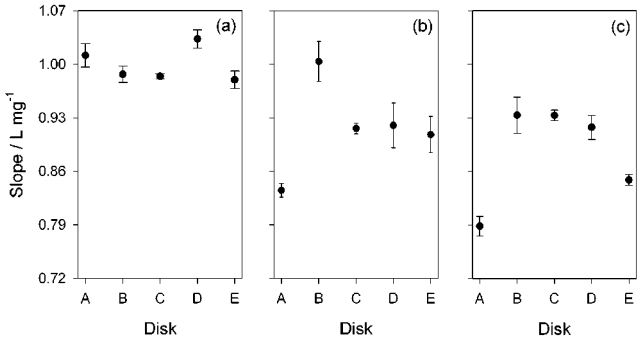

Figure 4. Slope of the relative luminescence intensity plots vs the oxygen concentration in water for five different O_2-sensitive disks, (a) uncoated and coated with (b) PC1008 or (c) PC1036 polymers.

polymer is shown to bond to poly(dimethylsiloxane)-based materials strongly enough to allow prolonged sensor application in aqueous environments.

The precision of the sensors, given as relative standard deviation for six measurements of five different oxygen concentrations taken over 18 h, is displayed in Table 1. PC polymer coating of the dyed silicone layers does not seem to affect the precision of the oxygen measurements. Repeatability of the data collected with the oxygen optodes is ± 0.1 mg L^{-1}. From the calibration curves, detection limits of 0.03, 0.04, and 0.05 mg L^{-1} for the uncoated, PC1008-, and PC1036-coated, respectively, have been obtained at 25 °C. These analytical parameters are similar to those displayed by commercial Clark-type oxygen sensors (e.g., the WTW's Oxy340A model we used for validation of the optode). Moreover, we have tested the reproducibility in the sensor fabrication (dyed substrate preparation plus PC polymer coating). The oxygen sensitivity data measured for five different disks (4-mm diameter) pierced from a single 75 × 25 mm membrane sheet are displayed in Figure 4. A comparison of the slopes of the calibration plots for each sensor demonstrates that statistically significant differences are obtained. The manual procedure of preparation of the silicone/silica gel mixture and subsequent film casting must be responsible for such variation, because incorporation of the indicator is made by equilibration of the cured membrane with a solution of the dye. It has been demonstrated that the oxygen sensitivity of the luminescent sensors is a function of the dyed silicagel loading level.[26] This effect has been ascribed to the combined effect of the higher O_2 uptake (for a given partial pressure) of the silicone films containing a higher amount of filler and the microsecond lifetime of the adsorbed indicator dye. The sorption−desorption kinetics of the oxygen molecule is faster than excited state deactivation of the Ru complex immobilized onto the filler surface, so that statistically, the higher the silica gel

Table 2. Response Time[a] of the Uncoated and PC-coated Luminescent Sensors to Different Oxygen Concentration Step Changes[b]

step (mg L^{-1})	uncoated	PC1008	PC1036
0.0 → 1.4	3.8 (8.4)	5.6 (10.0)	4.7 (11.1)
0.0 → 2.8	2.3 (9.5)	4.0 (13.0)	2.6 (11.8)
0.0 → 4.2	1.6 (9.8)	3.0 (14.3)	2.0 (12.8)
0.0 → 5.6	1.5 (10.6)	3.8 (15.8)	1.8 (14.4)
0.0 → 7.4	1.4 (10.8)	2.2 (17.2)	2.0 (14.6)
1.4 → 2.8	2.6 (4.3)	5.8 (9.7)	4.0 (6.0)
2.8 → 4.2	3.0 (3.8)	6.0 (8.8)	4.5 (5.0)
4.2 → 5.6	2.7 (3.8)	6.0 (9.8)	4.0 (6.0)
5.6 → 7.4	2.8 (4.1)	7.0 (9.0)	4.7 (5.5)

[a] t_{90}, min. [b] The response time for the corresponding reverse change is given in parentheses.

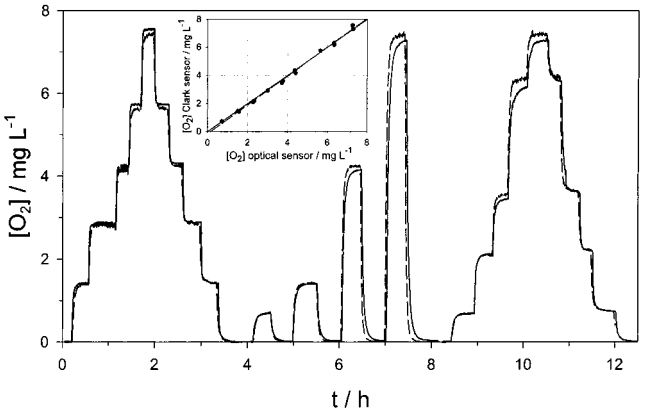

Figure 5. Simultaneous measurement of dissolved oxygen in water at 25.0 °C using the PC1036-coated luminescent optosensor (– – –) and a Clark-type electrode (—). Inset: correlation plot between the measured values using both techniques and theoretical (unity slope) line.

loading, the larger the number of quencher molecules that will be accessible to the luminescent indicator.[26] This effect would be similar to the well-known photoreactions of micelle-incorporated luminophores and quencher species bound in the Gouy−Chapman layer of the micelles.[28] Therefore, each oxygen optode must be individually calibrated unless the current membrane fabrication procedure can be improved.

The response is fully reversible and hysteresis has not been found. The sensor response times (t_{90}) at 25 °C for different step changes in the oxygen concentration are gathered in Table 2. In general, the indicator layers display the fastest response (1−2 min) for the largest upward changes in the oxygen level (0−7.5 mg L^{-1}), whereas their response time is similar for small step changes as it corresponds to smaller concentration gradients. The thin PC polymer coating slightly increases both the up and down t_{90} of the original luminescent membranes. The PC1036-coated layers respond faster to the analyte concentration changes than do their PC1008 analogues. Shorter response times would be obtained for thinner silicone layers, becaue the PC polymer coating (ca. 0.1 μm thick) makes a negligible contribution to the overall membrane thickness (ca. 200 μm). In this regard, we have found that the t_{90} decreases 2-fold when the thickness of the sensitive membrane

(28) Kalyanasundaram, K. *Photochemistry in Heterogeneous Systems*; Academic Press: Orlando, FL, 1987; pp 97−102.

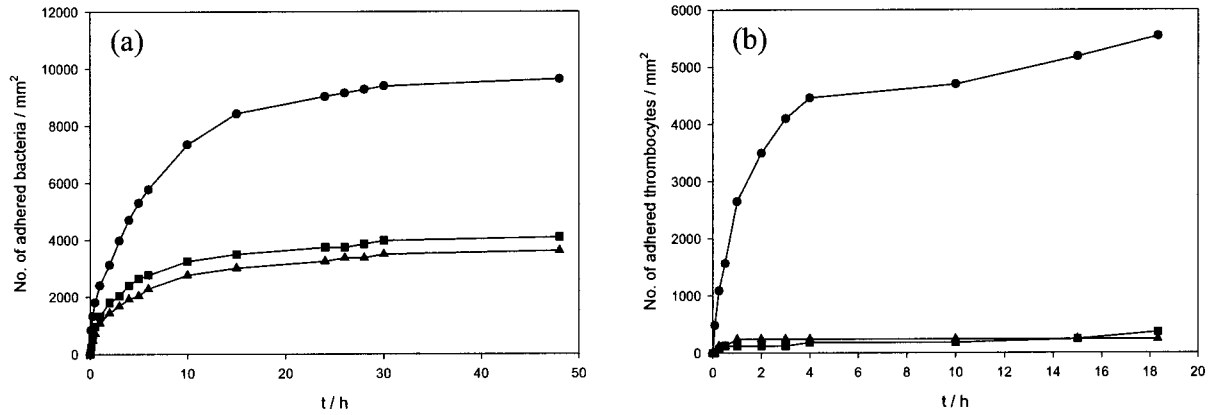

Figure 6. (a) *T. denitrificans* and (b) thrombocyte adhesion kinetics on uncoated (●), PC1008- (▲), and PC1036-coated (■) O_2-sensitive silicone films. The experimental points are mean values with 13% RSD.

is cut to ca. 50% of those reported in Table 2. The asymmetry of the up and down response times is associated with the differential oxygen sorption and desorption kinetics, as demonstrated by Mills and Chang.[29] Such thick membranes are required to prepare (manually) membrane sheets of an appropriate size for protein and cell adhesion studies (see below), but it might be readily decreased if required for environmental, medical, or industrial applications of the developed optodes.

The temperature effect on the oxygen sensitivity of the RD3-doped luminescent membranes has been investigated, too. The uncoated and the two types of PC-coated layers showed a similar behavior in the evaluated 5−35 °C range. The slope of the calibration plot increases linearly with increasing sample temperature: $(1.8 \pm 0.1) \times 10^{-2}$ L mg^{-1} °C^{-1} ($r = 0.9989$) for the PC1008-coated membranes and $(1.7 \pm 0.1) \times 10^{-2}$ L mg^{-1} °C^{-1} ($r = 0.9998$) for the PC1036-coated analogues. For the sake of comparison, the uncoated oxygen sensor shows a temperature coefficient of $(2.0 \pm 0.1) \times 10^{-2}$ L mg^{-1} °C^{-1} ($r = 0.9991$). The overall temperature effect is the result of opposite factors: (i) the solubility and adsorption properties of O_2 in the silica gel-filled silicone membrane, (ii) the O_2 diffusion into the poly(dimethylsiloxane), and (iii) the emission lifetime and O_2 quenching constant of the Ru(II) luminescent indicator. The oxygen solubility in RTV poly(dimethylsiloxanes) slightly decreases with increasing temperature ($S = S_0 e^{0.3/RT}$),[30] but the oxygen diffusion coefficient in the same medium slightly increases with increasing temperature ($D = D_0 e^{-9.0/RT}$).[30] Adsorption of gases onto pyrogenic silica depends inversely on temperature, according to the BET isotherm.[31] The kinetics of RD3 emission decay and O_2 quenching accelerate with increasing temperature.[32] Because (^{3}MLCT) excited state deactivation via thermally activated population of the nonradiative ^{3}MC state has little importance for Ru(II)-diphenylphenanthroline complexes (ca. 7% decrease of the emission lifetime from 278 to 308 K),[33] the temperature effect on the O_2 quenching must be the predominant factor that accounts for the observed increase of the sensor sensitivity with temperature.

The novel oxygen optodes have been validated using an accepted technique (the electrochemical Clark sensor) and calibrated electronic mass-flow meters with pure gases. Figure 5 depicts the response of the PC1036-coated luminescent optode for different step changes in the 0−8 mg L^{-1} O_2 concentration range as well as the correlation plot with the corresponding electrode readings ($y = (-0.16 \pm 0.11) + (1.03 \pm 0.02)x$; $r = 0.9986$). Similar response functions have been measured with the oxygen-sensitive membrane covered with PC1008 polymer (data not shown), with a correlation plot $y = (-0.11 \pm 0.08) + (1.04 \pm 0.02)x$ ($r = 0.9993$). The electrochemical sensor responds faster than the PC1008-coated optosensor for any oxygen concentration change, but the PC1036-coated material outperforms the Clark-type device for large-step changes (Figure 5). In any case, the correlation curves show no statistically significant differences between the oxygen readings obtained with both devices.

Bioadhesion Studies. Microorganism and thrombocyte adhesion onto the oxygen-sensitive films was investigated in order to evaluate the biofouling protection imparted by the novel PC polymer coatings. A mixed culture of typical marine denitrifying bacteria was used to simulate a marine environment toward the application of biofoul-proof optical sensors in the field of water monitoring. Cell adhesion experiments were carried out using a flow-through chamber and optical microscopy (see the Experimental Section and Figure 2). Image analysis showed a significant slowing of *T. denitrificans* adhesion kinetics onto PC-coated films, as compared to the uncoated silicone layers (Figure 6). At equilibrium, PC1008 and PC1036 have demonstrated to decrease by 70% the surface concentration of adhered bacteria. The higher PC contents of the former material, particularly as expressed at the interface, might account for the slightly stronger protection imparted by this anti-biofouling polymer. Figure 6 also displays platelet adhesion tests onto the surface of both the uncoated and PC-coated oxygen-sensing layers. It can be observed that platelets adhere to silicone faster than *Thiobacillus*: just 4 h is needed to reach saturation of the former onto the sensor surface. However, more than a 90% reduction in the number of adhered thrombocytes was found during the complete experimental time (18 h) on both the PC-1008- and PC-1036-coated silicone layers that were tested (Figure 2, insets). These observations have been confirmed by scanning electron and atomic force microscopies (results not shown). Moreover, preliminary experiments have demonstrated

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that the PC polymer coatings are also able to avoid adhesion of proteins onto the O_2-sensitive layers. A ca. 1-h incubation time for plasma with an uncoated silicone membrane yields 47.0 and 5.5 ng cm^{-2} of adsorbed fibrinogen and albumin, respectively. Under the same conditions, the PC1008 coating reduces adsorption of fibrinogen by 92% and adsorption of albumin by 64%. Therefore, PC polymer coatings would provide anti-biofouling protection to luminescent oxygen sensors for in vivo and bioprocess monitoring applications.

CONCLUSIONS

In summary, deposition of a nontoxic, biocompatible polymer coating based on phosphorylcholine is a suitable technology to impart highly effective anti-biofouling properties to luminescent oxygen optodes. The thin coatings perturb slightly or not at all the analytical features of these sensors. Such biomimetic materials will help to extend the operational lifetime of optical sensors in the application fields of environmental monitoring, clinical chemistry, and biotechnology.

ACKNOWLEDGMENT

This work has been funded by EU Industrial and Materials Technology (BriteEuRam III) Program (grant no. BRPR-CT97-0485, "BOSS" project) and the Spanish Government agency CICYT (equipment cofunding grant no. MAT98-1153-CE). The authors thank Dr. David Ayala for preparing some of the oxygen-sensitive films used in this study.

Received for review April 30, 2001. Accepted August 21, 2001.

AC015517N