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Response behavior of sodium-selective electrodes modified by surface attachment of the anticoagulant polysaccharides heparin and chondroitin sulfate

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Abstract

Two different polysaccharides with anticoagulant activities, heparin and chondroitin sulfate, were used to modify the surface of sodium-selective electrodes based on asymmetric cellulose triacetate (CTA) membranes. The membranes were formulated with sodium ionophore X, anionic additive, and *o*-nitrophenyl octyl ether. The response behavior of the surface-modified sodium electrodes was compared with that of control CTA, as well as poly(vinyl chloride) (PVC)-based sodium-selective electrodes. It was found that the selectivity coefficients obtained with the surface modified CTA membrane electrodes were slightly higher than those of the control, but in the case of heparin-modified electrodes they still met the requirements for analysis of sodium in physiological fluids within an error of <1%; the corresponding error for chondroitin sulfate-modified electrodes was also <1% except for the case of potassium ion in which the error was 1.3%. Likewise, it was found that other response characteristics, such as detection limit, linear range, slope of the response plot, selectivity pattern, and response time were comparable in both the control and the polysaccharide-modified electrodes. Therefore, the surface modification does not significantly alter the response behavior of the sensors.

Keywords: Membrane electrodes; Cellulose triacetate; Heparin; Chondroitin sulfate; Ion-selective electrodes; Biocompatibility

1. Introduction

Ion-selective electrodes (ISEs) are among the most commonly used electrochemical devices because of their simplicity, sensitivity, and high selectivity. Furthermore, they can be used for the direct and rapid measurement of various cations, anions, some gases, and polyions [1,2]. By far, the most important application of ISEs is in clinical and physiological analyses. As reported in a recent review [3], determination of the levels of blood electrolytes (e.g., sodium, potassium, calcium, magnesium, chloride, and pH) is the most frequently requested analysis in clinical laboratories. Further, the need for timely blood electrolyte determinations in emergency rooms and intensive care units has led to the development of short turn around time (stat) instruments for near patient testing [4–6]. The ideal solution to timely electrolyte determination would be their continuous in vivo monitoring in blood by implantable ISEs [4]. However, clotting on the surface of the ISE membrane presents itself as an obstacle. Therefore, the success of in vivo applications depends to a large extent on the biocompatibility of the sensors [7,8]. The most popular type of polymer used for the construction of membrane ISEs is poly(vinyl chloride) (PVC), which is particularly susceptible to clotting [9]. Blood clot formation on the surface of the electrode is not only undesirable medically, but also affects the overall response of the ISE. Thus, there is a need for the development of polymer matrices

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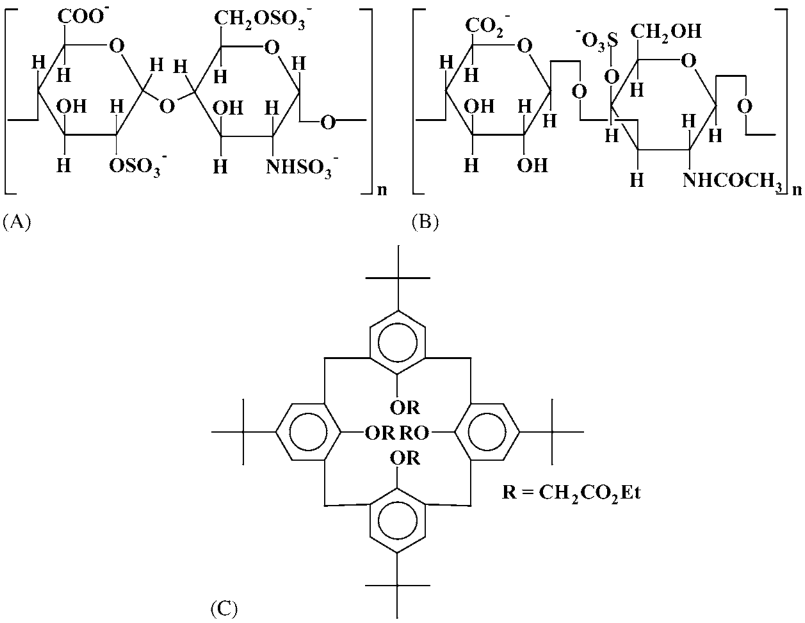

Fig. 1. Chemical structure of (A) heparin, (B) chondroitin sulfate, and (C) sodium ionophore X.

that are compatible with ISEs, yet prevent the formation of clots.

Any device introduced into a physiological medium induces biological reactions [8]. For example, upon introduction of an ion-selective electrode into blood or plasma, proteins readily adsorb onto the surface. This protein adsorption is the first step leading to several biological events, including activation of the coagulation cascade, cell adhesion, activation of platelets, and thrombi formation. Several strategies have been applied to manipulate these interactions that led to the invention of more biocompatible materials. For example, polymer materials with an inherent low affinity for protein adsorption and cell attachment have been introduced [10,11]. Another class of biomaterials has been developed through modification of polymeric material with molecules (e.g., heparin and poly(ethylene oxide)) that prevent platelet aggregation or decrease protein adsorption [12–16].

Some of the materials mentioned above are suitable for the preparation of sensors and have been adapted for the construction of more biocompatible ISEs [17]. For example, polymers that have been used in implantable devices, such as polyurethane [18], silicon rubber [19], and silicon rubber modified with hydrophobic end groups [20], have been employed as polymeric matrices for the design of ISEs. Surface coating of the sensors using biocompatible polymer coatings has been another approach to enhance the biocompatibility of sensors [21]. For instance, a biocompatible hydrogel containing phosphorylcholine groups, poly(2-methacryloyloxyethyl phosphorylcholine-co-butylmethacrylate) or poly(MPC-co-BMA), was used as a polymer coating to enhance the biocompatibility of potentiometric and amperometric sensors [22,23]. Membrane electrodes with improved biocompatibility were also prepared by blending poly(vinyl chloride) with a hydrophilic polymer such as poly(ethylene oxide) [24]. A recent successful approach to enhance the biocompatibility of sensors was introduced by Meyerhoff's group and is based on doping the polymeric matrix with NO-releasing compounds [25–27]; the released NO inhibits platelet aggregation.

Previously, we have shown that potassium-selective electrodes can be prepared by grafting heparin, an anticoagulant agent, onto the surface of cellulose triacetate membranes, impregnated with valinomycin. It was found that this surface modification had little effect on the response characteristics of the sensors and led to a more biocompatible potassium membrane electrode [28]. In this work, we extend our previous attempts by attaching polysaccharide anticoagulants on electrode surfaces and investigate their effect on the response behavior of sodium-selective electrodes. Two anticoagulant polysaccharides were used to modify the surface of cellulose triacetate sodium-selective electrodes, namely heparin and chondroitin sulfate. The effect of polysaccharide attachment on the potentiometric response characteristics was evaluated and compared with the unmodified CTA-based, as well as PVC-based sodium-selective electrodes.

2. Experimental

2.1. Reagents

Sodium ionophore X, heparin, chondroitin sulfate, and carbonyldiimidazole (CDI) were obtained from Sigma (St. Louis, MO) (see Fig. 1 for the chemical structures of sodium ionophore X, heparin, and chondroitin sulfate). Cellulose triacetate and chromatographic grade poly(vinyl chloride) were from Eastman Kodak (Rochester, NY) and Polysciences (Warrington, PA), respectively. Tetrahydrofuran (THF) was purchased from Fisher

(Fair Lawn, NJ) and tris(hydroxymethyl)aminomethane (Tris) from Research Organics (Cleveland, OH). *o*-Nitrophenyl octyl ether (NPOE) and potassium tetrakis[3,5-bis-(trifluoromethyl)phenyl]borate (KTFPB) were obtained from Fluka (Ronkonkoma, NY). Chloroform, methylene chloride, 1,1,2,2-tetrachloroethane, and all chloride salts were purchased from Aldrich (Milwaukee, WI). All standard solutions and buffers were prepared with deionized water (Milli-Q, Millipore, Bedford, MA).

2.2. Preparation of membranes

Cellulose triacetate unmodified (control) membranes were constructed by preparing first a base layer [28–30]. This layer was obtained by casting a solution containing 74 mg of CTA in 1.1 mL of methylene chloride, 0.40 mL of chloroform, and 0.40 mL of 1,1,2,2-tetrachloroethane in a 31 mm i.d. glass ring placed on a glass plate. After 2 days, the membrane was removed from the glass mold, and an ionophore-containing cocktail (composed of 1 mg of sodium ionophore X, 100 μL of *o*-NPOE, 35 mg of CTA, and 0.40 mg of KTFPB, all dissolved in a mixture of 0.80 mL of methylene chloride and 0.80 mL of chloroform) was poured into a 22 mm i.d. ring that was placed on top of the base layer. The solvent was allowed to evaporate for 2 days as the two layers fused into a single membrane.

Asymmetric hydrolyzed CTA control membranes were prepared by first constructing the base layer of the membrane as explained above. The membrane, which has raised edges at the area of contact with the glass ring, was removed from the mold and floated for 4.5 h in 1.0 M NaOH. Because of the raised edges, only one of the surfaces of the membrane was in contact with the NaOH solution. After this hydrolysis step, the membrane was rinsed with deionized water. The second layer, containing the active membrane components, was then added to the non-hydrolyzed side as described above.

Asymmetric polysaccharide-coated CTA membranes were prepared first by constructing the hydrolyzed layer as described above. Then, the hydrolyzed side of the membranes was immersed in cold deionized water, and 324 mg of solid CDI was added in five increments over a 15-min-period, giving a final concentration of 0.10 M CDI. Following this activation period, the membranes were immediately incubated overnight in a 1% (w/v) polysaccharide solution in 1.0 M sodium carbonate, pH 10, to promote covalent polysaccharide attachment. The modified membranes were rinsed sequentially with 0.10 M $NaHCO_3$ (pH 8.5), deionized water, acetate buffer (pH 4.0), and deionized water. Finally, the second layer was added to the unmodified side of the membranes as described above.

2.3. Construction of the membrane electrodes and measurement setup

Small disks of 7 mm diameter were cut from the master membrane as well as the control asymmetric membranes (i.e., with (a) non-hydrolyzed CTA and (b) hydrolyzed CTA, but without polysaccharide attachment) and mounted onto electrode bodies (Philips IS-561; Glasblaserei Möller, Zurich), with the polysaccharide-modified surface facing the sample solution. The internal filling solution was 0.010 M NaCl. A double-junction Ag/AgCl electrode (Orion Model 90-02-00) with an Orion (90-02-02) internal filling solution was used as the reference electrode. The outer compartment of the reference electrode was filled with 0.100 M Tris–HCl, pH 7.5. All potentiometric measurements were performed in 0.100 M Tris–HCl, pH 7.5. Membrane potentials were monitored with an in-house custom-built four channel high-impedance amplifier with unity gain, coupled to an analog-to-digital converter (G.W. Instruments; Somerville, MA) connected to a Macintosh computer using SuperScope V. 1.2 (G.W. Instruments) software. The detection limit, linear range, and selectivity coefficients were determined as in references [31–33], and the data presented in this paper are an average of three ion-selective electrode measurements.

3. Results and discussion

The biocompatibility of sensors is inherently related to the properties of the sensor material that comes in contact with physiological fluids. Attachment of anticoagulation agents to the surface, coating the surface with a biopolymer, or doping the sensors with reagents that release compounds that interfere with the blood clotting cascade have been found to be useful strategies in enhancing the biocompatibility of sensors. Previously, we have shown that heparin modification of CTA-based potassium-selective electrodes did not alter significantly the response behavior of the electrodes [28]. In this study, we investigated the effect of immobilization of anticoagulant polysaccharides (heparin and chondroitin sulfate) on the response characteristics of CTA-based sodium-selective electrodes.

The response behavior of four sets of membranes (CTA, hydrolyzed CTA, heparin-modified CTA, and chondroitin sulfate modified CTA) was compared. Fig. 2 shows the potentiometric response of sodium-selective electrodes based on sodium ionophore X and unmodified CTA toward various cations. The electrodes showed a sodium detection limit of 3.2×10^{-5} M and had a linear response range of 1.4×10^{-4} to 0.1 M. The CTA electrodes had a higher detection limit than the corresponding poly(vinyl chloride)-based sodium-selective electrodes formulated with sodium ionophore X and the same plasticizer (Table 1). In addition, the slope obtained with the CTA-based sodium-selective electrodes was lower than that of the corresponding PVC-based electrodes [34]. Similar results were obtained for the previously reported CTA-based potassium membrane electrode formulated with valinomycin as ionophore and NPOE as plasticizer [28]. Sodium electrodes based on PVC and surface-modified with poly(ethylene oxide) also showed a decrease in the response slopes compared to unmodified PVC elec-

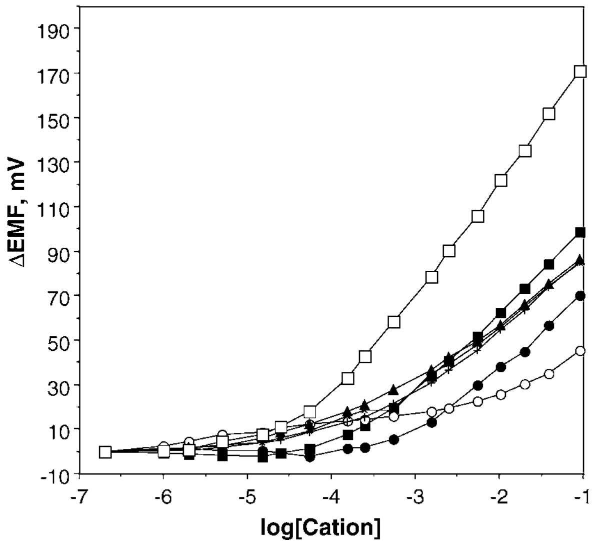

Fig. 2. Selectivity pattern of unmodified CTA membrane electrodes using sodium ionophore X as the ionophore. The cations tested are (□) sodium, (■) potassium, (▲) cesium, (×) rubidium, (+) ammonium, (●) lithium, and (○) magnesium.

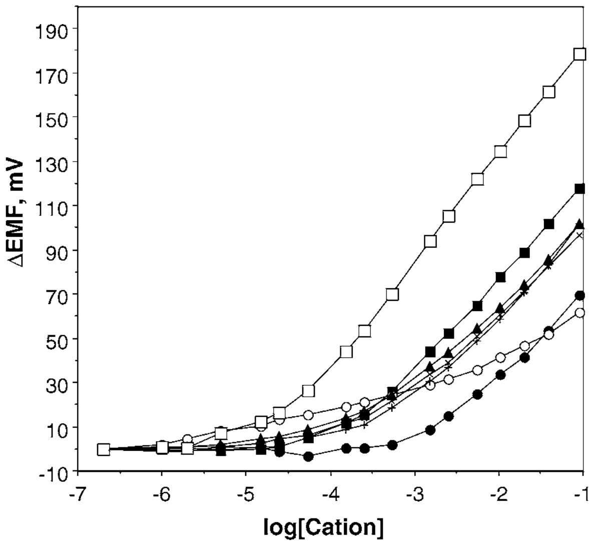

Fig. 3. Selectivity pattern of hydrolyzed CTA membranes. For a key to the symbols associated with each calibration plot, see legend to Fig. 2.

trodes [24]. Nonetheless, the physiological range for sodium ion lies within the linear response range of the CTA-based membrane electrodes. For comparison purposes, the selectivity coefficients of CTA-based sodium membrane electrodes were calculated using the separate solution method at 0.1 M cation concentration. As shown in Table 1, changing the polymeric matrix from PVC to CTA worsens the selectivity of the electrodes. The selectivity sequence, however, remains practically the same for both sodium electrode systems. Moreover, the selectivity coefficients of both systems meet the requirements for sodium analysis in physiological fluids with <1% error (see data in Table 1) [35].

The potentiometric response behavior of sodium electrodes based on hydrolyzed CTA membranes was evaluated to probe the effect of leftover hydroxyl groups that may remain on the surface after attachment of the polysaccharides. A comparison of Figs. 2 and 3 (data summarized in Table 1) reveals that the selectivity sequence and the selectivity coefficients of both the hydrolyzed and the non-hydrolyzed CTA-based electrodes remained practically unchanged. This indicates that any leftover hydroxyl groups after the polysaccharide immobilization would not interfere with the electrode response.

Previously, we have shown that attachment of heparin to the surface of CTA-based potassium-selective electrodes, formulated with valinomycin, has practically no effect on the potentiometric response characteristics [28]. To prove that this approach for improving the biocompatibility of neutral carrier-based membrane electrodes can be extended to other cation electrodes, we studied the effect of heparin attachment on the response behavior of sodium-selective electrodes based on CTA and formulated with sodium ionophore X. The immobilization conditions employed result in 0.16 μg of attached heparin per mg of membrane [28]. Coupling takes place through CDI-reactive functional groups on heparin, while maintaining biological activity [28]. As can be seen in

Table 1
Summary of the response characteristics of sodium membrane electrodes

Polymeric matrix	$\log K^{\text{pot.}}_{\text{Na}^+,\text{cation}}$							Slope (mV/decade)	Detection limit (M)	Linear range (M)
	Na^+	K^+	Cs^+	Rb^+	NH_4^+	Li^+	Mg^{2+}			
PVC[a]	0	−2.0	NA	NA	−2.7	−2.5	−2.9	60	3.5×10^{-6}	NA
CTA	0	−1.5	−1.7	−1.7	−1.7	−2.0	−2.5	50	3.2×10^{-5}	1.6×10^{-4} to 0.10
Hydrolyzed CTA	0	−1.2	−1.6	−1.7	−1.6	−2.2	−2.4	49	2.0×10^{-5}	1.6×10^{-4} to 0.10
Heparin-modified CTA	0	−1.0	−1.2	−1.1	−1.3	−1.2	−2.0	45	6.3×10^{-5}	1.6×10^{-4} to 0.10
Chondroitin sulfate modified CTA	0	−0.44	−1.3	−1.2	−1.3	−1.3	−2.3	46	5.6×10^{-5}	1.6×10^{-4} to 0.10
Required selectivity coefficient[b]	0	<−0.6	NP	NP	<1.6	<−0.1	<−1.2	–	–	–

NA, data not available; NP, not applicable.

[a] From [34].

[b] Required selectivity coefficient for blood analysis (1% error; worst case); [35].

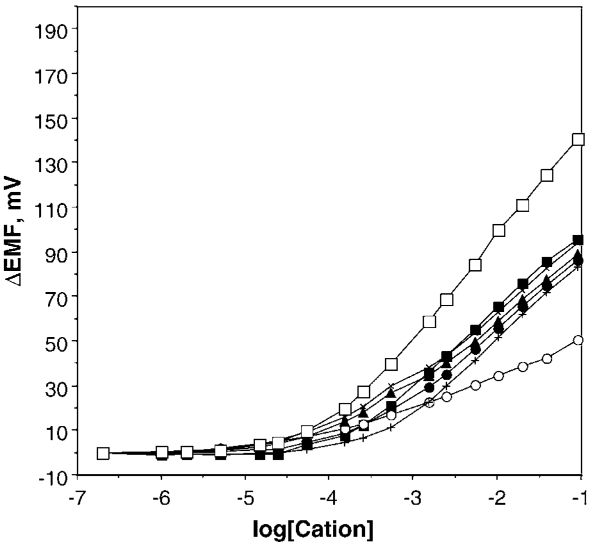

Fig. 4. Selectivity pattern of heparin-coated CTA membrane electrodes. For a key to the symbols associated with each calibration plot, see legend to Fig. 2.

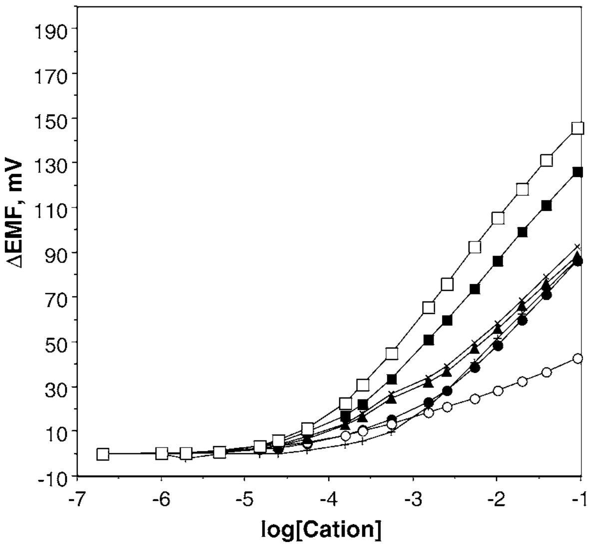

Fig. 5. Selectivity pattern of chondroitin sulfate coated membrane electrodes. For a key to the symbols associated with each calibration plot, see legend to Fig. 2.

Fig. 4, sodium electrodes based on CTA and surface-modified with heparin maintained practically the same selectivity sequence as that of hydrolyzed or non-hydrolyzed CTA membrane electrodes. This reinforces our previous finding that heparin immobilization does not significantly change the response behavior of ion-selective electrodes. The selectivity coefficients of the heparin-modified electrodes were slightly higher than those of the unmodified-CTA membrane electrodes. Similar results were obtained for heparin-modified potassium electrodes based on CTA and formulated with valinomycin [28]. It is worth mentioning that the selectivity coefficients obtained with heparin-modified CTA-based electrodes fulfill the requirements for the analysis of sodium in physiological fluids within 1% error for all the tested cations (see data in Table 1) [35].

Chondroitin sulfate is a naturally occurring substance, which is present in body tissue. Both in vitro and in vivo studies showed that chondroitin sulfate has antithrombotic effects, which lead to prolongation of the blood clotting time [36–39]. Therefore, similar to heparin, chondroitin sulfate immobilization should enhance the biocompatibility of the CTA-based membrane electrodes. As can be seen in Fig. 5, electrodes based on CTA modified with chondroitin sulfate showed a selective sodium response with a slope and detection limit very close to those of heparin-modified sodium electrodes (Table 1). Furthermore, sodium-selective electrodes based on CTA and surface modified with chondroitin sulfate had a selectivity pattern similar to that of heparin-modified electrodes. The selectivity coefficients obtained with chondroitin sulfate modified membranes were found to meet the requirement for the analysis of sodium in physiological fluids with less than 1% error for all cations except for potassium for which the error would be 1.3%. The response times obtained for the unmodified CTA, hydrolyzed CTA, heparin-modified CTA, and chondroitin sulfate modified CTA electrodes were found to be in the order of 1–2 min depending on the sodium ion concentration in the sample solution (Fig. 6). This response time is comparable with the response time obtained with PVC-based membranes indicating that the surface polysaccharide layer does not hinder the diffusion of sodium within the time frame of the experiment.

In summary, two anticoagulant polysaccharides, heparin and chondroitin sulfate, were used to modify the surface of CTA-based sodium electrodes. The response behavior of the polysaccharide-modified CTA membrane electrodes (e.g., detection limit, linear range, response slope, and selectivity coefficients) was comparable to that of PVC-based sodium electrodes. The modified membrane electrodes were found to

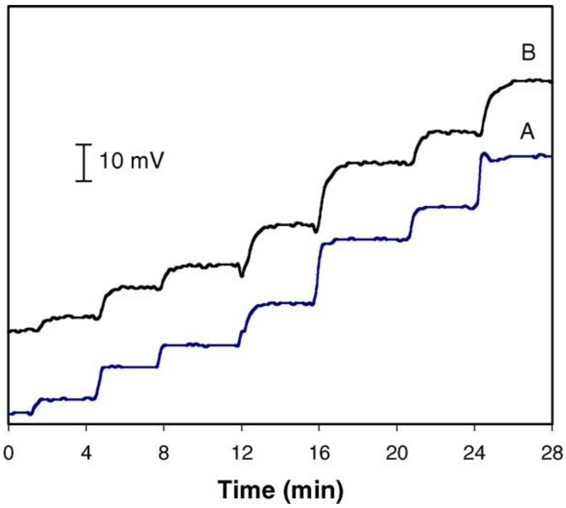

Fig. 6. Dynamic response profiles of asymmetric membranes with surface attached (A) heparin and (B) chondroitin sulfate. An offset of 20 mV was applied to the response pattern of the chondroitin sulfate membrane electrode shown in this figure for clarity.

retain their selectivity sequence; the selectivity coefficients, although higher than those of PVC electrodes, still fulfilled the requirement for sodium analysis in physiological fluids (with an error of <1.3%). The asymmetric CTA membrane system could be adapted for the development of catheter type sensors using dip-coating techniques. The immobilization of heparin to enhance the biocompatibility of traditional polymers, such as polyurethane and poly(dimethylsiloxane), has been reported in the literature (see, for example, [40,41]) and could be adapted to enhance the biocompatibility of sensors based on those polymers.

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