Electrochemical Sensor for Catechol and Dopamine Based on a Catalytic Molecularly Imprinted Polymer–Conducting Polymer Hybrid Recognition Element

8 AUTHORS, INCLUDING:

Dhana Lakshmi
18 PUBLICATIONS 443 CITATIONS

SEE PROFILE

Alessandra Bossi
University of Verona
83 PUBLICATIONS 2,305 CITATIONS

SEE PROFILE

Michael Whitcombe
University of Leicester
103 PUBLICATIONS 3,719 CITATIONS

SEE PROFILE

Sreenath Subrahmanyam
University of Aberdeen
33 PUBLICATIONS 849 CITATIONS

SEE PROFILE

Electrochemical Sensor for Catechol and Dopamine Based on a Catalytic Molecularly Imprinted Polymer-Conducting Polymer Hybrid Recognition Element

Dhana Lakshmi, Alessandra Bossi,[†] Michael J. Whitcombe,* Iva Chianella, Steven A. Fowler, Sreenath Subrahmanyam, Elena V. Piletska, and Sergey A. Piletsky

Cranfield Health, Vincent Building, Cranfield University, Cranfield, Bedfordshire MK43 0AL, U.K.

One of the difficulties with using molecularly imprinted polymers (MIPs) and other electrically insulating materials as the recognition element in electrochemical sensors is the lack of a direct path for the conduction of electrons from the active sites to the electrode. We have sought to address this problem through the preparation and characterization of novel hybrid materials combining a catalytic MIP, capable of oxidizing the template, catechol, with an electrically conducting polymer. In this way a network of "molecular wires" assists in the conduction of electrons from the active sites within the MIP to the electrode surface. This was made possible by the design of a new monomer that combines orthogonal polymerizable functionality; comprising an aniline group and a methacrylamide. Conducting films were prepared on the surface of electrodes (Au on glass) by electropolymerization of the aniline moiety. A layer of MIP was photochemically grafted over the polyaniline, via *N,N'*-diethyldithiocarbamic acid benzyl ester (iniferter) activation of the methacrylamide groups. Detection of catechol by the hybrid-MIP sensor was found to be specific, and catechol oxidation was detected by cyclic voltammetry at the optimized operating conditions: potential range −0.6 V to +0.8 V (vs Ag/AgCl), scan rate 50 mV/s, PBS pH 7.4. The calibration curve for catechol was found to be linear to 144 μM, with a limit of detection of 228 nM. Catechol and dopamine were detected by the sensor, whereas analogues and potentially interfering compounds, including phenol, resorcinol, hydroquinone, serotonin, and ascorbic acid, had minimal effect ($\leq$3%) on the detection of either analyte. Non-imprinted hybrid electrodes and bare gold electrodes failed to give any response to catechol at concentrations below 0.5 mM. Finally, the catalytic properties of the sensor were characterized by chronoamperometry and were found to be consistent with Michaelis−Menten kinetics.

For reasons of their recognition properties, stability, reproducibility, low cost, robustness, and manufacturing potential, molecu-larly imprinted polymers (MIPs)[1,2] are regarded as promising candidates for the replacement of biomolecules as the recognition element in a range of chemical sensors.[3] This research has seen MIPs used with a number of transducer technologies, including piezoelectric elements,[4,5] optical methods,[6,7] and in electrochemical sensing.[8−10] The pace of development of MIP-based sensors however, especially in the area of electrochemical sensing, has not matched the expectations of what appears to be a promising technology. This may be an indication that the integration of MIPs with sensor technology is not as straightforward as one might expect.

The first generation of MIP sensors was prepared using imprinted polymers synthesized in the form of monoliths. These were required to be ground and sieved to prepare MIP particles which were then deposited in close proximity to the electrode; for example, by the incorporation of the particles into the carbon paste of screen-printed electrodes,[11] or into a supporting gel prepared with agarose.[12] It was observed that the response of the sensor was strongly dependent on how intimately the MIP and transduction element were integrated and how efficient the electrical "communication" was between them. The size of the MIP particles strongly influenced the response time of the sensors,[13−15]

* To whom correspondence should be addressed. E-mail: m.whitcombe@cranfield.ac.uk. Phone: +44 1234 758329.
† Dipartimento Scientifico e Tecnologico, Università di Verona, Strada Le Grazie 15, 37134 Verona, Italy.

(1) Wulff, G. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 1812–1832.
(2) Alexander, C.; Andersson, H. S.; Andersson, L. I.; Ansell, R. J.; Kirsch, N.; Nicholls, I. A.; O'Mahony, J.; Whitcombe, M. J. *J. Mol. Recognit.* **2006**, *19*, 106–180.
(3) Hillberg, A. L.; Brain, K. R.; Allender, C. J. *Adv. Drug Delivery Rev.* **2005**, *57*, 1875–1889.
(4) Lieberzeit, P. A.; Dickert, F. L. *Anal. Bioanal. Chem.* **2008**, *391*, 1629–1639.
(5) Uludag, Y.; Piletsky, S. A.; Turner, A. P. F.; Cooper, M. A. *FEBS J.* **2007**, *274*, 5471–5480.
(6) Stephenson, C. J.; Shimizu, K. D. *Polym. Int.* **2007**, *56*, 482–488.
(7) Al-Kindy, S.; Badía, R.; Suárez-Rodríguez, J. L.; Díaz-García, M. E. *Crit. Rev. Anal. Chem.* **2000**, *30*, 291–309.
(8) Kriz, D.; Mosbach, K. *Anal. Chim. Acta* **1995**, *300*, 71–75.
(9) Malitesta, C.; Losito, I.; Zambonin, P. G. *Anal. Chem.* **1999**, *71*, 1366–1370.
(10) Blanco-López, M. C.; Lobo-Castañón, M. J.; Miranda-Ordieres, A. J.; Tuñón-Blanco, P. *Trends Anal. Chem.* **2004**, *23*, 36–48.
(11) Sode, K.; Takahashi, Y.; Ohta, S.; Tsugawa, W.; Yamazaki, T. *Anal. Chim. Acta* **2001**, *435*, 151–156.
(12) Kröger, S.; Turner, A. P. F.; Mosbach, K.; Haupt, K. *Anal. Chem.* **1999**, *71*, 3698–3702.
(13) Kirsch, N.; Hart, J. P.; Bird, D. J.; Luxton, R. W.; McCalley, D. V. *Analyst* **2001**, *126*, 1936–1941.
(14) Pizzariello, A.; Stred'ansky, M.; Stred'anska, S.; Miertuš, S. *Sens. Actuators B* **2001**, *76*, 286–294.

indicating that miniaturization of the particles responsible for analyte recognition would be an efficient strategy.[16] A parallel approach, which may help to overcome some of these limitations, involved the preparation of MIP-based recognition elements as layers or thin films deposited or grafted onto the transducer surface.[17−20] In the case of thin films the sensor response showed a dependency on the film thickness. For acrylic polymers, the effective range of thickness was found to be around 10−100 nm.[21]

It should be noted, however, that one of the recognized characteristics of acrylic polymers is their ability to serve as electrical insulators; this is clearly an undesirable property as far as their incorporation into electrochemical sensors is concerned and poses severe limitations on the efficiency of the signal transduction process. What is required is a direct path for the conduction of electrons from the binding sites to the electrode, which is lacking in conventional MIP-based systems. A significant advance, therefore, was the use of polymers that possess intrinsic electron-transport properties, imprinted with templates, as the basis of selective electrochemical sensors.[22] Imprinting of templates has been carried out in films of poly(pyrrole),[23−25] poly-(aniline),[26] or poly(aminophenyl boronic acid)[27] for example, prepared through chemical oxidation or electrochemical polymerization on the surface of electrodes. MIPs prepared from organic semiconductors offer advantages in terms of preparation of the chemosensitive layer (such as fine control of layer thickness by electropolymerization, reproducibility, and ease of manufacture) and in terms of signal transduction; nevertheless, "classical" imprinted polymers, especially those based on acrylic or vinylic monomers, offer a much wider variety of functional groups and thus a greater choice of suitable polymerizable monomers and cross-linkers able to address a broader range of templates.

The integration between MIPs and polymeric semiconductors is a way to take advantage of the properties of both materials. The polymerization of pyrrole within the pores of a MIP was reported to have no detrimental effect on the recognition abilities of the MIP;[28] however, despite this original paper, the idea has not been further explored. The entrapment within or anchoring of MIP particles onto electrosynthesized poly(pyrrole) films was also proposed as a solution to improve the integration between MIPs and transducer.[29] This allowed the composite film to be grown potentiostatically, with benefits for sensor manufacturing.

In addition it allowed for optimization of the polymerization conditions, both for the MIP and for the semiconducting polymer layer separately. The results of this study demonstrated that the approach was promising and showed a high level of selectivity in the sensor response. More recently, other electrically conducting materials such as carbon nanotubes have been employed at the interface between the electrode and MIP.[30]

In the present paper, we propose a simpler approach to create closer integration between a MIP and transducer. Here we report the development of a sensor based on a novel hybrid material, prepared from N-phenylethylene diamine methacrylamide (NPEDMA). The newly designed monomer, NPEDMA, combines orthogonal polymerizable functionalities, comprising an aniline group and a methacrylamide function, each of which can be independently polymerized.[31] Electropolymerization of NPEDMA therefore results in the formation of a layer of poly(aniline) at the electrode surface with pendant double bonds capable of participating in an addition polymerization. In this way MIPs can be grafted in intimate contact with the substituted poly(aniline) layer, which therefore can act as a network of "molecular wires", facilitating the conduction of electrons from the recognition sites in the MIP to the electrode surface. In the first demonstration of this approach, conducting films were prepared on the surface of gold electrodes by electropolymerization, followed by photochemical grafting of the MIP. This was achieved by N,N′-diethyldithiocarbamic acid benzyl ester (iniferter) activation of the methacrylamide groups on the electropolymerized layer before addition of the MIP components. The MIP chosen for this study had previously been developed within our group as a tyrosinase-mimicking polymer, imprinted with catechol.[32] Catalytic MIP conducting polymer hybrid electrodes were prepared and characterized, and the results are presented below. We also report the sensor characteristics, reproducibility, sensitivity, linearity, and selectivity of the sensor with respect to catechol, dopamine, and potential interfering substances. Finally, the catalytic activity for catechol was assessed using data obtained by chronoamperometry.

EXPERIMENTAL PART

Materials and Methods. Catechol, resorcinol, hydroquinone, (-)-epinephrine, norepinephrine, serotonin hydrochloride, dopamine, sodium benzoate, ethylene glycol dimethacrylate (EGDMA), N-phenyl ethylenediamine, methacrylic anhydride, acetonitrile, dimethylformamide, perchloric acid, and methanol were purchased from Sigma-Aldrich (Gillingham, U.K.). Phenol and resorcinol were obtained from Fluka (Gillingham, U.K.). 1,2-Naphthalenediol, 1,4-naphthalenediol, and iniferter were purchased from TCI (Oxford, U.K.). All the other chemicals were of analytical

(15) Yamazaki, T.; Meng, Z.; Mosbach, K.; Sode, K. *Electrochemistry* **2001**, *69*, 969–972.

(16) Ye, L.; Cormack, P. A. G.; Mosbach, K. *Anal. Chim. Acta* **2001**, *435*, 187–196.

(17) Hedborg, E.; Winquist, F.; Lundström, I.; Andersson, L. I.; Mosbach, K. *Sens. Actuators A* **1993**, *37–38*, 796–799.

(18) Sergeyeva, T. A.; Piletsky, S. A.; Brovko, A. A.; Slinchenko, E. A.; Sergeeva, L. M.; Panasyuk, T. L.; El'Skaya, A. V. *Analyst* **1999**, *124*, 331–334.

(19) Yoshimi, Y.; Ohdaira, R.; Iiyama, C.; Sakai, K. *Sens. Actuators B* **2001**, *73*, 49–53.

(20) Syu, M. J.; Chiu, T. C.; Lai, C. Y.; Chang, Y. S. *Biosens. Bioelectron.* **2006**, *22*, 550–557.

(21) Panasyuk-Delaney, T.; Mirsky, V. M.; Ulbricht, M.; Wolfbeis, O. S. *Anal. Chim. Acta* **2001**, *435*, 157–162.

(22) Lange, U.; Roznyatovskaya, N. V.; Mirsky, V. M. *Anal. Chim. Acta* **2008**, *614*, 1–26.

(23) Deore, B.; Chen, Z. D.; Nagaoka, T. *Anal. Sci.* **1999**, *15*, 827–828.

(24) Özcan, L.; Sahin, Y. *Sens. Actuators B* **2007**, *127*, 362–369.

(25) Ramanavicius, A.; Ramanaviciene, A.; Malinauskas, A. *Electrochim. Acta* **2006**, *51*, 6025–6037.

(26) Sreenivasan, K. *J. Mater. Sci.* **2007**, *42*, 7575–7578.

(27) Deore, B.; Freund, M. S. *Analyst* **2003**, *128*, 803–806.

(28) Kriz, D.; Andersson, L. I.; Khayyami, M.; Danielsson, B.; Larsson, P. O.; Mosbach, K. *Biomimetics* **1995**, *3*, 81–90.

(29) Mazzotta, E.; Picca, R. A.; Malitesta, C.; Piletsky, S. A.; Piletska, E. V. *Biosens. Bioelectron.* **2008**, *23*, 1152–1156.

(30) Kan, X. W.; Zhao, Y.; Geng, Z. R.; Wang, Z. L.; Zhu, J. J. *J. Phys. Chem. C* **2008**, *112*, 4849–4854.

(31) Lakshmi, D.; Whitcombe, M. J.; Davis, F.; Chianella, I.; Piletska, E. V.; Guerriero, A.; Subrahmanyam, S.; Brito, P. S.; Fowler, S. A.; Piletsky, S. A., *Chem. Commun.* **2009**, DOI: 10.1039/b821409c.

(32) Piletsky, S. A.; Nicholls, I. A.; Rozhko, M. I.; Sergeyeva, T. A.; Piletska, E. V.; El'Skaya, A. V.; Karube, I. *Ukr. Biokhim. Zh.* **2005**, *77*, 63–67.

grade and were used as received. Ultrapure water (Millipore) was used for analysis. NMR spectra were obtained using a JEOL ECX-400 multinuclear NMR spectrometer. NMR solvents were obtained from Goss Scientific (Chelmsford, U.K.). Gold coated glass SPR slides (2 cm × 2 cm, with 45 nm evaporated Au film over 5 nm Cr) were obtained from NanoSPR, (U.S.A.) and used to prepare the working electrodes. The gold-coated slides were cut in half to form two 1 cm × 2 cm slides. A strip of epoxy adhesive (Araldite) was used to separate a working electrode of 1 cm × 1 cm from the area used to make electrical contact. Static water contact angle measurement was performed using a Cam 100 optical Angle Meter (KSV Instruments Ltd., Finland). Atomic Force Microscopy (AFM) images were obtained in contact mode using the PicoScan instrument (Molecular Imaging Corporation, Tempe, AZ, U.S.A.).

Urocanic Acid Ethyl Ester. This compound was prepared according to the literature procedure for the synthesis of the methyl ester.[33] To a solution of E-urocanic acid (1.5 g, 10.86 mmol) in HPLC-grade ethanol (20 mL) was added anhydrous sodium sulfate (0.225 g) and concentrated sulfuric acid (0.9 mL). The suspension was heated to reflux with stirring for 14 h. The reaction mixture was filtered to remove sodium sulfate and concentrated under reduced pressure. The resulting solid was dissolved in 10 mL of water and neutralized by the addition of saturated sodium bicarbonate at 0 °C until evolution of gas ceased. Extraction with ethyl acetate, drying of the organics over sodium sulfate, and evaporation under reduced pressure yielded the product as a white solid that was used without further purification. ^{1}H NMR, δ, (CDCl$_3$) 1.31 (t, 3H), 4.23 (q, 2H), 6.47 (d, 1H), 7.28 (s, 1H), 7.60 (d, 1H), 7.70 (s, 1H). ^{13}C NMR, δ, (CDCl$_3$) 14.31, 60.38, 116.23, 136.78, 167.48.

N-Phenylethylene Diamine Methacrylamide (NPEDMA). N-phenylethylenediamine (1.0 g, 0.96 mL, 7.3 mmol) was dissolved in methanol (20 mL) which was cooled in ice before the addition of methacrylic anhydride (1.1 g, 1.06 mL, 7.1 mmol). The stirred mixture was held at 0 °C for 3 h before warming to room temperature. The solvent was removed using a rotary evaporator, and the residue dispersed in diethyl ether (25 mL). The ether phase was washed with 0.1 M NaOH, (4 × 25 mL) followed by water (1 × 25 mL), dried over anhydrous magnesium sulfate, and evaporated to give a brown oil which formed colorless crystals on standing in a refrigerator. The yield was 99%. ^{1}H NMR, δ, (CD$_3$Cl) 1.9 (s, 3H, -CH$_3$), 3.3 (d, 2H, -CH$_2$), 3.7 (d, 2H, -CH$_2$), 5.3 (s, 1H, =CH$_2$), 5.7 (s, 1H, =CH$_2$), 6.6 (d, 2H, ortho-H), 6.7 (t, 1H, para-H), 7.2 (m, 2H, meta-H). ^{13}C NMR, 18.6, 39.3, 43.9, 112.7, 117.7, 119.9, 129.3, 139.7 147.9, 169.0. FT-IR (KBr disk) 3345, 3000, 2920, 1655, 1603, 1514, 1322, 750, 694 cm^{-1}.

Electropolymerization of NPEDMA. Electrochemical experiments were performed on an Autolab PSTAT 10 instrument (Eco-Chemie BV, Utrecht, Netherlands) using a conventional three-electrode cell with the modified 1 cm × 1 cm gold slide as working electrode, a platinum electrode as an auxiliary electrode, and an Ag/AgCl reference electrode. Cyclic voltammetry (CV) was performed between −0.2 V and +1.0 V (vs Ag/AgCl) for 15 cycles at a scan rate of 50 mV s^{-1} in a solution of NPEDMA (2.44 mM) in HClO$_4$ (50 mM). The gold electrode bearing the electropo-

lymerized polyaniline film with pendant methacrylamide groups was washed with water and dried.

Immobilization of Iniferter to Pendant Double Bonds of the Poly(NPEDMA) Layer. A solution of diethyl dithiocarbamic acid benzyl ester in acetonitrile (10 mL, 0.63 mM) was prepared in a 10 cm glass Petri dish. The electropolymerized gold thin film electrode was immersed in the iniferter solution with the polymer layer uppermost. After the removal of oxygen by purging with argon for 10 min, the Petri dish was covered with a flat glass plate using Parafilm to form a seal. A UV lamp delivering 9 W cm^{-2} (Philips home tanning lamp) was mounted at a distance of 8 cm from the surface of the bench and used to irradiate the contents of the Petri dish for 20 min. The electrode was then washed with methanol and dried in a stream of argon gas.

Grafting of Molecularly Imprinted Polymer to the Iniferter-Activated Poly(NPEDMA) Layer. A solution of urocanic acid ethyl ester (62.3 mg, 375 μmol), catechol (6.9 mg, 62.5 μmol) and CuCl$_2$ (134.45 mg, 1000 μmol) was prepared in DMF (2.5 g) in a 30 mL capacity vial, followed by the addition of ethyleneglycol dimethacrylate (1.20 g, 6.06 mmol). The reaction mixture was sonicated for 10 min. The iniferter-activated polymer-coated electrode was immersed in the reaction mixture in a small glass Petri dish. After removal of oxygen by purging with argon for 4 min, the Petri dish was covered with a flat glass plate, sealed using Parafilm, as above. UV irradiation delivering 9 W cm^{-2} (Philips home tanning lamp) mounted at 8 cm from the surface of the bench was carried out for 30 min to graft MIP to the electrode surface. A similar procedure was also followed to prepare the (non-imprinted) control-grafted electrodes. In this case the same steps were carried out with the exclusion of template (catechol) from the polymerization mixture. Template removal was carried out by washing the electrode with aliquots of EDTA solution (0.1 M) until the electrochemical signal for catechol, as monitored by CV, disappeared (5−6 washes). Electrodes were then rinsed with DMF, followed by water and dried in air. Control (non-imprinted) electrodes were subjected to the same washing conditions used with the MIP material.

X-ray Photoelectron Spectroscopy (XPS). Measurements were carried out on a VG ESCA laboratory-Mark-2 X-ray Photoelectron Spectrometer (East Grinstead, U.K.). The X-ray gun was operated at 14 kV and 20 mA. Survey and high resolution spectra were collected at 50 and 100 eV respectively, with Mg Kα 1253.6 eV radiation. The vacuum in the analysis chamber was about 10^{-9} mBar. High resolution scans with a good signal ratio have been obtained in the C1s, N1s, O1s, and S2p regions of the spectrum. The decomposition of the XPS peaks into different components and the quantitative interpretation have been performed after subtraction of the background using the Shirley method.[34]

Cyclic Voltammetry (CV). Electrochemical experiments were performed using an Autolab PSTAT 10 instrument (Eco-Chemie BV, Utrecht, Netherlands) with the polymer-modified working electrode, a platinum auxiliary electrode, and an Ag/AgCl reference electrode. Polymer-modified electrodes were immersed in PBS, pH 7.4 (5 mL) containing CuCl$_2$ (5 mM), for 5 min, after which the electrodes were washed with buffer alone. The copper-loaded polymer electrode was attached to the instrument as working electrode and aliquots of a freshly prepared

(33) Richard, D. J.; Schiavi, B.; Joullié, M. M. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 11971–11976.

(34) Shirley, D. A. *Phys. Rev. B: Condens. Matter* **1972**, *5*, 4709.

Table 1. Effect of Structural Analogues and Potentially Interfering Compounds on the Anodic Peak Current Response for Catechol and Dopamine Detection in CV Measurements Carried out on the MIP-Hybrid Electrode[a]

analyte	analyte concentration (mM)	analogue/interferent	analogue/interferent concentration (mM)	anodic peak current (μA)	current ratio (i_{C+I}/i_C)[b]
catechol	0.11			30.9	1.0
		1,4-naphthalenediol	1.0	no peak	
catechol	0.11	1,4-naphthalenediol	1.0	30.5	0.99
		1,2-naphthalenediol	1.0	no peak	
catechol	0.11	1,2-naphthalenediol	1.0	31.1	1.01
		hydroquinone	1.0	no peak	
catechol	0.11	hydroquinone	1.0	30.8	1.0
		ascorbic acid	100	no peak	
catechol	0.11	ascorbic acid	100	31.1	1.01
		phenol	100	no peak	
		resorcinol	90	no peak	
catechol	0.11	ascorbic acid + phenol + resorcinol	100/100/90	31.68	1.03
dopamine	0.1			17.8	
dopamine	0.3			53.2	
		epinephrine	1	no peak	
dopamine	0.1	epinephrine	1	17.75	1.0
		norepinephrine	1	no peak	
dopamine	0.1	norepinephrine	1	17.7	0.99
		serotonin	1	no peak	
dopamine	0.1	serotonin	1	17.9	1.01

[a] Representative CV traces can be seen in the Supporting Information, Figures S6−S15. [b] i_C = peak current recorded with analyte alone; i_{C+I} = peak current recorded in the presence of analyte and analogue/interferent.

solution of catechol (4 μM−0.3 mM) in PBS, pH 7.4, were pipetted into the voltammetric cell. The cyclic voltammograms were obtained by scanning the potential from −0.6 to +0.9 V versus Ag/AgCl at a scan rate of 50 mV s⁻¹. A calibration curve was constructed using the standard addition method, correlating the catechol concentration with the anodic peak current, as determined by CV. To demonstrate the interelectrode repeatability, results were averaged for three separately fabricated electrodes and three measurements per electrode. The limit of detection was calculated as 3 times the standard deviation for the blank measurement (in the absence of catechol) divided by the slope of the calibration plot.[35]

Chronoamperometry. Kinetic data for catechol oxidation was obtained by chronoamperometry for polymer-modified electrodes at a potential of 0.3 V. Chronoamperograms were obtained for various concentrations of catechol (4 μM−0.3 mM) in PBS, pH 7.4. The working electrode was preconditioned at a potential of 0.3 V for 2 min before each measurement. The hybrid MIP electrode was dipped into a stirred solution of CuCl₂ (5 mM) in PBS for 2 min followed by washing with buffer. The copper-loaded electrode was then dipped into a stirred solution of catechol at the test concentration (in PBS, pH 7.4), the potential applied immediately, with sampling of the current performed at 0.3 s intervals. From plots of i versus t, the slopes for the initial 0.9 s were obtained. Data were fitted with the Michaelis−Menten model, and apparent kinetic parameters were calculated. The same experiments were repeated with the template analogue dopamine.

Optimization of the Sensor Construction. To determine the optimum conditions for the deposition of poly(NPEDMA), electrodes were prepared as described above but varying the number of electrochemical potential cycling steps during the electropolymerization stage. The number of cycles used was between 0

and 50. Electrodes were assessed for their sensor performance in the detection of catechol as a function of peak current (CV measurement) and rate of oxidation of catechol (chronoamperometry).

Analogues and Interfering Substances. The selectivity of the sensor to catechol and dopamine in the presence of analogues of the analytes and potentially interfering substances was also tested in CV. Cyclic voltammograms were obtained for analogues and interfering species alone and in the presence of 0.11 mM catechol or 0.1 mM dopamine (Table 1). CVs were recorded in PBS, pH 7.4. Peak detection was performed using the Autolab software (Eco-Chemie BV, Utrecht, Netherlands).

Determination of the Chronoamperometric Response to Catechol in the Absence of Oxygen. The copper-loaded MIP-hybrid electrode (as above) was sonicated for 5 min in buffer while the solution was purged with nitrogen. Similarly the test solution, consisting of catechol (0.11 mM) in PBS, was sonicated and simultaneously purged with nitrogen for 5 min before insertion of the electrode and recording of the chronoamperogram at a potential of 0.3 V.

Determination of the Quantity of Copper Binding Sites on the MIP-Hybrid Electrodes. Chronoamperometry on solutions of catechol (0.11 mM in PBS) was carried out on the MIP-hybrid electrodes, as above, except that the molar quantity of copper in the solution used to load the electrode with the metal ion was varied over the range 10−800 nmol. The slope over the initial 0.9 s (V_{max}) was plotted against copper concentration.

Reproducibility and Storage Stability of the Catalytic Performance of the MIP-Hybrid Electrodes. Chronoamperograms for catechol solutions (0.11 mM in PBS) were recorded, as described above, using the same electrode periodically over a period of 2 months, and the slopes obtained compared. When the sensor was not in use, the electrode was kept in the dry state in a covered Petri dish at room temperature.

(35) Skoog, D. A.; Holler, F. T.; Nieman, T. A. *Principles of Instrumental Analysis*, 5th ed.; Harcourt Brace College Publishers: Orlando, FL, 1998.

Scheme 1. Preparation of the Catalytic MIP-Hybrid Electrodes Imprinted with Catechol
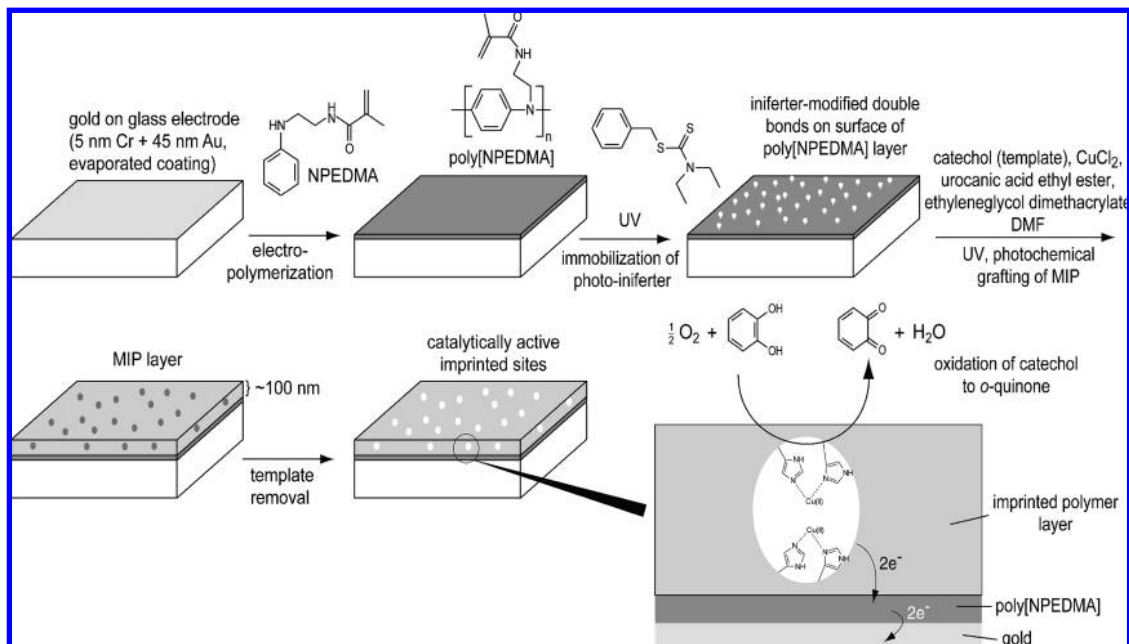
RESULTS AND DISCUSSION

Preparation and Characterization of Catalytic MIP-Conducting Polymer Hybrid Electrodes. The MIP hybrid sensor was constructed as indicated in Scheme 1. The monomer, NPEDMA, which incorporates orthogonal polymerizable groups in the form of the aniline and methacrylamide functionalities, was used to form the interface between the gold electrode and the MIP catalyst. This monomer was originally designed for this work, although it has many other potential applications which are currently being investigated and will be discussed elsewhere.[31] The starting point for electrode preparation was therefore the electrochemical deposition of a film of poly(NPEDMA) onto gold-coated glass electrodes. The number of CV cycles used in the deposition of an electropolymerized layer will affect the thickness and morphology of the deposited film[36] and is also likely to affect the sensor performance. The number of deposition cycles was therefore varied over the range 0–50 and the sensor response to catechol (0.11 mM), in terms of both the peak current and the rate of the oxidation reaction, was compared (Figure 1). From this study, 15 cycles, over the potential range −0.2 V and +1.0 V (vs. Ag/AgCl), was judged to be optimal.

To facilitate the grafting of the MIP or non-imprinted control polymer, the pendant double bonds derived from the methacrylamide moiety of the poly(NPEDMA) scaffold were activated by photochemical coupling with iniferter, which acts as a "living" free radical initiator,[37] allowing grafting of MIP or control through subsequent polymerization steps.[38,39] To prepare the hybrid catalytic sensor, a catalytic MIP was photochemically grafted to the iniferter-activated poly(NPEDMA) network. The polymer chosen for this purpose was a substrate-imprinted mimic of the enzyme tyrosinase, previously prepared within the group,[32] which was active in the two-electron oxidation of *o*-diphenols to

o-quinones, characteristic of the enzyme.[40] The mimic of the active site was obtained by the template-induced placements of 4–6 imidazole-containing residues (derived from urocanic acid ethyl ester) which provide coordination sites for two copper ions, recreating the dinuclear copper center. Catechol was used as template in MIP-grafted electrodes; control-grafted electrodes were prepared in the same way in the absence of template.

Electrodes were characterized during their preparation by AFM, by XPS, and by static water contact angle measurements. The contact angles were found to be 66° for bare gold, 31° for the poly(NPEDMA) network, 74° after iniferter attachment, and 53° after catalytic MIP grafting, which are strongly suggestive that the surface chemistry has changed at each stage of electrode preparation. The surface topography, as measured by AFM, is shown in the Supporting Information, Figure S1. The poly-
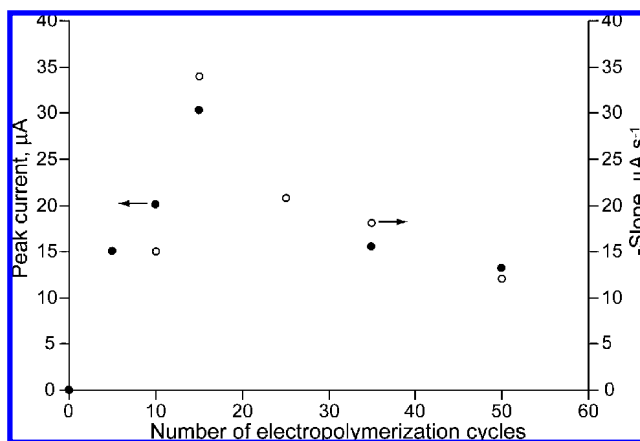

Figure 1. Optimization of the number electropolymerization cycles used in the deposition of the poly(NPEDMA) layer with respect to the sensor response for 0.11 mM catechol. Data is presented both for the peak current obtained from CV measurements and from the initial slopes obtained from chronoamperometry. A clear peak in sensitivity is seen for electrodes prepared using 15 scans. This value was used for all electrode preparations.

(36) Shim, H.-S.; Yeo, I.-H.; Park, S.-M. *Anal. Chem.* **2002**, *74*, 3540–3546.
(37) Otsu, T. *J. Polym. Sci. A, Polym. Chem.* **2000**, *38*, 2121–2136.
(38) Sellergren, B.; Rückert, B.; Hall, A. J. *Adv. Mater.* **2002**, *14*, 1204–1208.
(39) Pérez-Moral, N.; Mayes, A. G. *Macromol. Rapid Commun.* **2007**, *28*, 2170–2175.
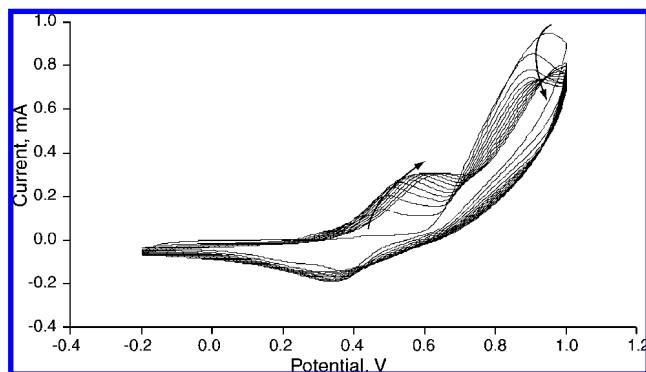
Figure 2. Cyclic voltammograms obtained during the electropolymerization of NPEDMA (2.44 mM) in $HClO_4$ (50 mM), scans 1−12. CV was performed between −0.2 V and +1.0 V (vs. Ag/AgCl).

(NPEMA) layer (Supporting Information, Figure S1A) shows similar contours to the grain structure of the underlying gold surface (not shown). The grafted MIP layer also shows a similar morphology (Supporting Information, Figure S1B) with a somewhat smoother appearance.

A set of XPS measurements was made to confirm the expected changes in surface chemistry during the construction of the MIP-hybrid sensor. For poly(NPEDMA), survey scans showed the presence of oxygen (538 eV), nitrogen (407 eV), gold (359 and 341 eV), a doublet peak for gold (88 eV), and again at 62 eV. Following iniferter immobilization, new peaks due to sulfur (154 and 168 eV) were observed, confirming the attachment of the dithiocarbamate-based iniferter groups at the surface. (XPS spectra are presented in the Supporting Information, Figure S2.)

Electrochemical Behavior of the MIP-Hybrid Sensor. The electropolymerization behavior of the NPEDMA layer was observed by CV, and a typical series of scans (1−12) are shown in Figure 2. It is likely that oxidation of the aniline part occurs close to +1.0 V (marked with the descending arrow in Figure 2). A secondary oxidation peak (marked by the ascending arrow in Figure 2) is absent in the first scan and is probably due to intermediate species formed during the polymerization, as has been observed in the electropolymerization of other anilines.[41]

When the MIP is grafted to the poly(NPEDMA) scaffold, the recognition/catalytic element should co-exist with the conducting network. To prove whether the process of hybrid electrode construction can generate connections due to "molecular wires", enabling the formation of pathways for the exchange of electrons between the catalytic center and the electrode, the CV of the MIP-grafted surfaces was explored in the presence of substrate (catechol) and is reported in Figure 3. This compound is both the template used in the construction of the MIP sites, as in the original MIP design,[32] and is a substrate for the enzyme tyrosinase. In addition, the ortho-dihydroxyphenyl structural motif is characteristic of a number of physiologically important molecules such as the catecholamines. Experiments were carried out on copper-loaded electrodes in the presence of air. In the presence of 0.11 mM catechol a CV peak was observed at +0.35 V with the MIP-hybrid electrodes (Figure 3A) but not with the non-imprinted-hybrid (control) electrodes (Figure
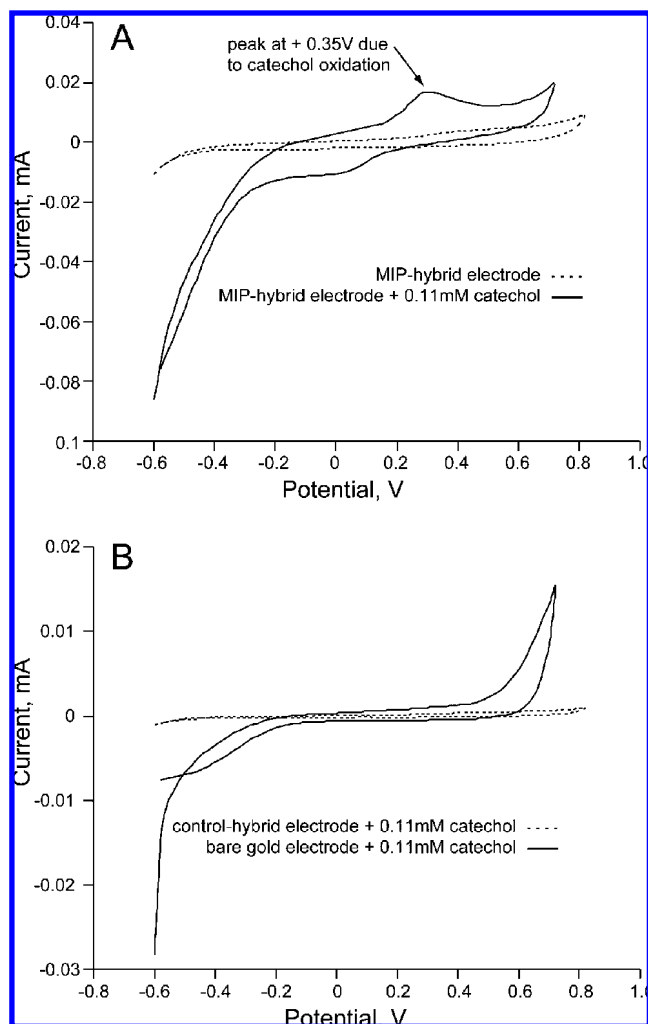

Figure 3. Comparison between the electrochemical behaviors of MIP- and control-hybrid electrodes and bare gold electrode (after loading with 5 mM copper chloride in the case of polymer-hybrid electrodes) as determined by CV (PBS pH 7.4, scanning at 50 mV s^{-1}, −0.6 to + 0.9 V versus Ag/AgCl) for (A) MIP-hybrid electrode (dashed line) and MIP-hybrid electrode in the presence of 0.11 mM catechol (solid line); and (B) non-imprinted control-hybrid electrode (dashed line) and bare gold electrode (solid line), both in the presence of 0.11 mM catechol.

3B) nor with a bare gold electrode (Figure 3B). CV experiments indicated that catalytic oxidation of catechol occurred only on the MIP-hybrid sensor. The appearance of a prominent reduction peak, with onset at around −0.4 V (Figure 3A), was observed on the MIP electrode in the presence of catechol, but not in its absence or on the bare gold electrode. The peak is reminiscent of that for the reduction of hydrogen peroxide[42] and may indicate that H_2O_2 is a byproduct of catechol oxidation on this polymer. The mechanism of action of catechol oxidase mimics is discussed by Koval et al.[43] which includes possible routes to the production of hydrogen peroxide. It is not within the scope of the present study to further elucidate the mechanism of action of the MIP catalyst; however, it is highly likely that catalysis within the MIP is not directly analogous to that occurring with the enzyme.
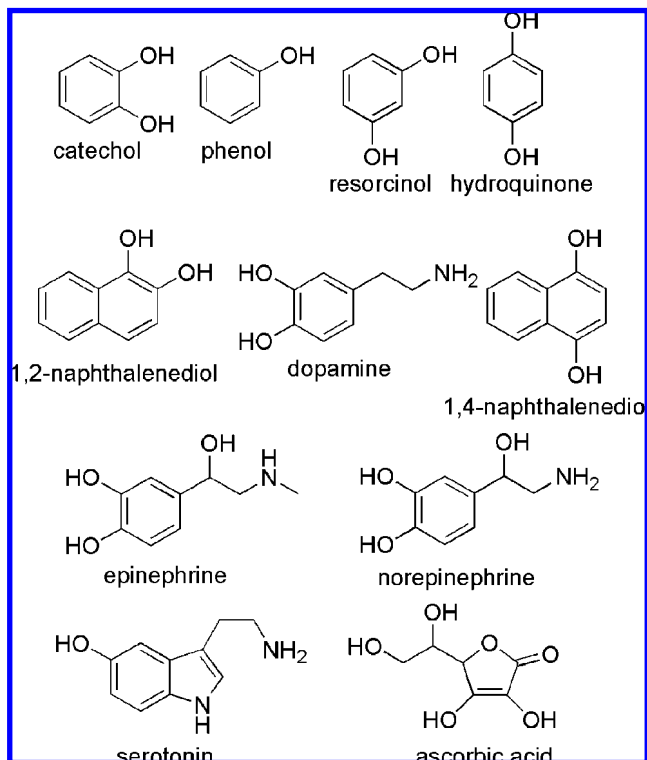
(40) Decker, H.; Schweikardt, T.; Tuczek, F. *Angew. Chem., Int. Ed.* **2006**, *45*, 4546–4550.
(41) Jin, M.; Yu, Z.; Xia, Y. *Russ. J. Electrochem.* **2006**, *42*, 964–968.
(42) Zhang, J. D.; Oyama, M. *Electrochim. Acta* **2004**, *50*, 85–90.
(43) Koval, I. A.; Gamez, P.; Belle, C.; Selmeczi, K.; Reedijk, J. *Chem. Soc. Rev.* **2006**, *35*, 814–840.

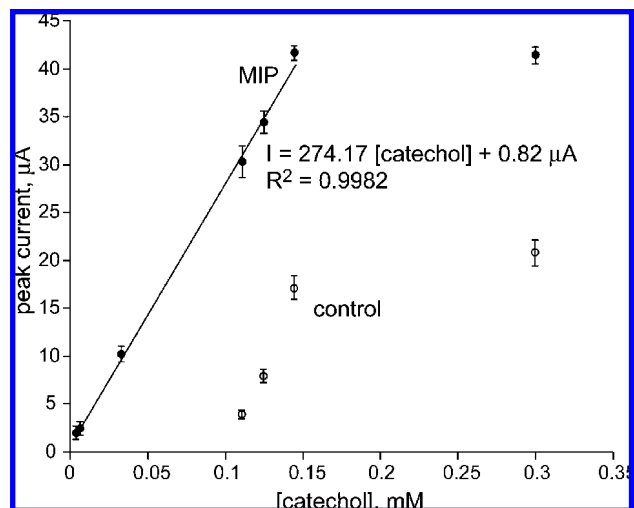**Scheme 2. Structures of the Compounds Used in This Study**

Figure 4. Calibration plot for the MIP-hybrid electrode of peak current versus catechol concentration and the corresponding data for the non-imprinted control-hybrid electrode. The limit of detection for catechol on the MIP-hybrid sensor was 228 nM. Data points were obtained as the average of three separate measurements made on three separate electrodes; error bars represent ± 1 standard deviation.

Additional evidence that a substrate-selective transformation of catechol or dopamine was taking place on the MIP-hybrid electrode came from the CVs of compounds structurally related to catechol or dopamine (Scheme 2) and their mixtures with either analyte: no peaks were detected for any of the compounds tested except for catechol and dopamine (Table 1). In the presence of most analogues, the recorded peak current diagnostic for catechol or dopamine oxidation was within 1% of the value obtained in the absence of interferent, showing that the sensor could be used quantitatively in mixtures with these compounds, present at higher concentrations. The only exception was for catechol (0.11 mM) detection in the presence of a mixture of phenol, resorcinol, and ascorbic acid, at 100, 90, and 100 mM concentrations, respectively, where the error rose to 3%. Phenol, resorcinol, hydroquinone, and 1,4-naphthalenediol and serotonin are all phenolic compounds; however, they lack the ortho-dihydroxy motif required for the formation of a complex with copper, which accounts for their lack of interaction with the MIP. It is less easy to explain why epinephrine and norepinephrine do not give rise to a sensor signal, given their structural analogy to dopamine. This could arise from a lower intrinsic propensity to oxidation or could be due to selectivity inherent in the imprinted polymer, which can readily accommodate a template analogue bearing a simple side chain but not the greater steric demand of a side-chain bearing an α-hydroxy substituent.

It was notable also that ascorbic acid, which is the most commonly interfering substance in the electrochemical detection of electroactive substances (such as catecholamines) in physiological fluids,[44] also did not interfere with catechol detection, even when present at 100 mM, which is around 10^3 times the

concentration of the analyte. It was also notable that dopamine could be detected in the presence of a 10-fold excess of serotonin, suggesting that sensors based on this approach could be used to monitor dopamine concentrations in physiological fluids.

Sensitivity Range of the MIP-Hybrid Catechol Sensor. A calibration curve for catechol detection on the MIP-hybrid sensor was determined. Data points were calculated as the average from three separate electrodes. Peak current versus catechol concentration (Figure 4) exhibited a linear response over a wide range of concentrations (over 3 orders of magnitude), from 228 nM to 144 μM. The limit of detection was 228 nM. No detectable signal was observed on non-imprinted control electrodes for catechol concentrations below 100 μM, clearly demonstrating the profound influence of the template in the formation of the selective binding site for catechol. These results and those above illustrate the great selectivity and sensitivity of the MIP-hybrid system and provide an interesting perspective for its application.

Catalytic Parameters of the MIP Hybrid Sensor. The apparent catalytic properties of the MIP-hybrid sensor were investigated through chronoamperometric measurements conducted in a forced convection system. The method allowed the MIP-hybrid catalyzed reaction to be monitored directly *in situ* without a lag-phase,[45] in a system based on the "diffusion" layer approach.[46] The steady-state limiting current is expressed by the equation:

$$I = \frac{NFAD_0 c_0^*}{\delta_0}$$

where A is the surface area of electrode, D_0 is the diffusion coefficient, c_0^* the bulk concentration of reactant, and δ_0 the

(44) Wolfrum, B.; Zevenbergen, M.; Lemay, S. *Anal. Chem.* **2008**, *80*, 972–977.

(45) Ikeda, T.; Kurosaki, T.; Takayama, K.; Kano, K. *Anal. Chem.* **1996**, *68*, 192–198.

(46) Bard, A. J.; Faulker, L. R. *Electrochemical Methods*, 2nd ed.; Wiley: New York, 2001.
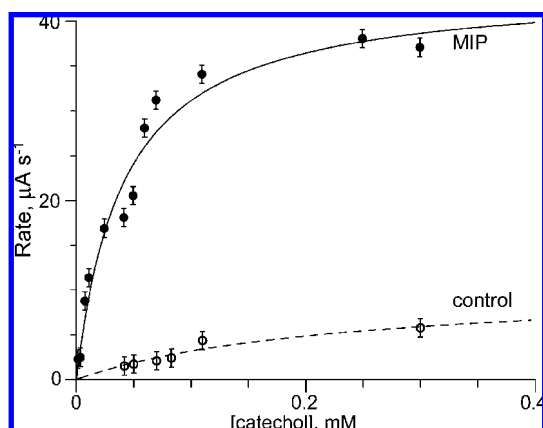
Figure 5. Plots of the reaction rate versus substrate (catechol) concentration, as determined by chronoamperometry at +0.3 V after the addition of catechol, for the MIP-hybrid and control electrodes. The MIP data shows a profile typical of the Michaelis–Menten model of enzyme kinetics.

"diffusion" layer thickness by convective transfer, this latter parameter depending on the stirring rate, diffusion coefficient, and viscosity.

The kinetic behavior of the MIP-hybrid material in the oxidation of catechol (0.11 mM) as substrate was determined. Catechol oxidation was treated as a pseudo-first order reaction, described by the apparent rate constant k. The rate of reaction on the MIP-hybrid electrodes was $k_{MIP} = 1.47 \times 10^{-3}$ min^{-1}. A comparison of this figure with those obtained for the non-imprinted control-hybrid electrodes and for oxidation on bare gold electrodes gave significant rate enhancement factors of $k_{MIP}/k_{control} = 44$ and $k_{MIP}/k_{bare} = 82$. Experiments also showed that the reaction did not occur in the absence of oxygen or when the MIP-coated electrode was not preloaded with copper ion. These data are both suggestive of a catalytic mechanism. No signal was observed under the same conditions with either resorcinol or phenol, again demonstrating the selective nature of the reaction.

The curve of initial rate (derived from the slope of current versus time over the first 0.9 s of chronoamperometric measurement) against substrate concentration was plotted in Figure 5. The curve follows the Michaelis–Menten profile, indicating an enzyme-like catalytic process. It should be emphasized, however, that catalysis at a surface layer with the consequent restrictions on diffusion may not fulfill the criteria for strict application of the Michaelis–Menten model, and this must be borne in mind when comparing this result with other published data.

In enzymes such as tyrosinase, the rate enhancement for the oxidation of substrate stems from the cumulative contributions of a number of different factors: the metal-mediated chemical step, the proximity effect, and the strain distortion. Here, the MIP-hybrid material seemed to possess some of these characteristics, with the exception of strain distortion. The absence of the latter effect can be attributed to the fact that the catalytic MIP was prepared using the substrate (catechol) as the template which serves as the transition state analogue in this material.[32,47]

The derived apparent Michaelis–Menten parameters for the oxidation of catechol and of the structural analogue dopamine on

(47) Wulff, G. *Chem. Rev.* **2002**, *102*, 1–27.

Table 2. Apparent Michaelis-Menten Parameters Obtained for the Oxidation of Catechol and Dopamine on the MIP-Hybrid Electrodes[a]

substrate	K_M (mM)	V_{max} (10^{-3} mM min^{-1})	k_{cat} (min^{-1})	k_{cat}/K_M (mM^{-1} min^{-1})	K_i (μM)
catechol	0.049	2.28	0.0456	0.931	0.34
dopamine	0.093	1.68	0.0333	0.359	n.d.[b]

[a] Sodium benzoate was used as a competitive inhibitor. [b] n.d., not determined.

the MIP-hybrid electrode are given in Table 2. In the case of catechol oxidation a value of $K_M = 0.049$ mM was obtained for reaction on the MIP-hybrid sensor (the corresponding value for the control-hybrid electrode being 0.185 mM, in addition the value of V_{max} was 5-fold lower on the control than the MIP). The result from the non-imprinted control polymer is accounted for by the presence of a random distribution of copper binding sites, only a minority of which exists in a catalytically active disposition. Presumably if both copper ion and the template were excluded from the synthesis of the control polymer, an even lower activity would be observed because of the random distribution of urocanic acid ethyl ester groups, which would result in a much reduced capacity to bind copper.

The turnover number, k_{cat} was calculated from the equation $V_{max} = k_{cat} \times$ [E]t. V_{max} was obtained experimentally from velocity measurement in the presence of substrate above the saturation concentration and was determined by chronoamperometry. [E]t indicates the concentration of catalytically active sites, which is derived from Et, the number of active sites within the polymer. This number was estimated from the number of copper ions complexed by the MIP layer, assuming that each active site contains two copper ions. This measurement was performed indirectly, by recording the initial rate of catechol oxidation (0.11 mM) in the presence of limiting quantities of copper (in the range 10−800 nmol) until saturation was observed. It was observed that the rate of catechol oxidation fell below the maximum when the quantity of copper in solution was less than ∼500 nmol, (see Supporting Information, Figure S3) giving a value of Et = 250 nmol as a working estimate of the number of active sites in 5 mL of solution (the sample volume), hence the concentration, [E]t, was calculated on this basis. The turnover number, k_{cat} was reasonably high on the MIP-hybrid electrode and clearly demonstrated that turnover was taking place.

Sodium benzoate, a competitive inhibitor for the enzyme, also inhibited the catalytic ability of the MIP-hybrid electrode. This was shown to be dependent on the concentration of the inhibitor and exhibited a value of K_i 100 times lower than the value of K_M (graphs used in the estimation of K_i are presented in the Supporting Information, Figures S4 and S5).

When dopamine was used as substrate for oxidation some interesting data were gathered. As expected, the value of K_M for dopamine was significantly higher (double) than that for catechol, indicating a lower affinity of the recognition sites for the non-template molecule. However, V_{max} and k_{cat} for dopamine were both around 73% of the values obtained for catechol, indicating the preference for catechol, nevertheless the MIP-hybrid did work as catalyst for dopamine oxidation. The
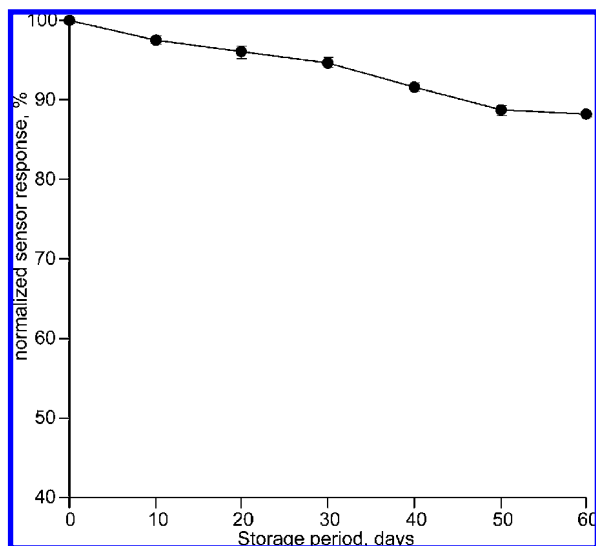
Figure 6. Storage stability of the MIP-hybrid electrode over 60 days. The error bars represent ± 1 standard deviation, $n = 3$.

catalytic performance, expressed in terms of k_{cat}/K_M, was three times higher in case of the template, than for dopamine. In general k_{cat}/K_M values are satisfactory for an enzyme mimic,[47] and similar numbers were obtained for both dopamine and catechol. The MIP-hybrid sensor was therefore an efficient catalyst of both catechol and dopamine oxidation and hence capable of sensing both analytes.

Storage Capacity of the MIP-Hybrid Sensors. The catalytic stability of the MIP-hybrid sensor over time was checked during a period of 2 months. In between measurements the sensor was kept at room temperature and in the dry state. A graph of V_{max}, normalized to the initial value, is presented in Figure 6. A small loss in catalytic activity (~10%) was measured after 60 days storage. These results indicate a significant stability of the sensor and capacity for repeated measurement to be performed on the same electrode.

CONCLUSIONS

The catalytic MIP-hybrid sensor presented here was based on the idea of providing a solution to the perennial problem of how to form an electrical connection from the recognition and/or catalytic sites within an insulating MIP to an electrode surface for efficient signal transduction. This was achieved by first laying down an electropolymerized film of NPEDMA, followed by iniferter activation and grafting of a previously known catalytic MIP composition. The MIP mimics the active site of the enzyme tyrosinase in which the copper-mediated oxidation of catechol occurs in the presence of atmospheric oxygen. The overall redox reaction results in the release of two electrons which gives rise to the signal at +0.35 V, as the active site copper ions are reoxidized. The vast improvement due to the poly(NPEDMA) layer can be seen by contrasting these results with our earlier efforts to integrate this MIP, prepared as ground particles produced from a monolith, with a screen printed electrode. In that case no signals were recorded, emphasizing the need for a conduction pathway between MIP sites and the working electrode.

While here we have postulated a polymer bilayer structure for the hybrid sensor, an interpenetrating network structure may have resulted instead. This would account for the relatively facile conduction of electrons from the catalytic sites to the gold surface and would result if the solvent (DMF) used during the MIP grafting step was able to swell the poly(NPEDMA) layer, producing catalytic sites throughout the thickness of the polymeric layer, rather than confining them to the vicinity of the surface.

The kinetic properties for the catalytic oxidation of catechol on the MIP appear to follow the Michaelis–Menten model, including the susceptibility to competitive inhibition, similar to the enzyme tyrosinase. Moreover the MIP hybrid sensor displayed remarkable sensitivity for catechol and an ability to detect dopamine even in the presence of various structural analogues and known interfering compounds; thus dopamine could be detected in the presence of serotonin, epinephrine, or norepinephrine while the presence of almost a thousandfold excess of ascorbic acid did not affect catechol detection.

The approach here proposed demonstrates a valid route for the preparation of a new generation of sensors based on conducting polymer hybrid materials, which are robust and sensitive, highly suitable for the electrochemical detection of a range of analytes. The chronoamperometric signal is generated within a second, and thus could be used for real time measurements.

SUPPORTING INFORMATION AVAILABLE

Additional information as mentioned in the main text. This material is available free of charge via the Internet at http://pubs.acs.org.