Published in final edited form as:
Anal Chem. 2008 June 15; 80(12): 4499–4517. doi:10.1021/ac8007219.

Electrochemical Sensors

Benjamin J. Privett[†], **Jae Ho Shin**[‡], and **Mark H. Schoenfisch**[†]

[†]Department of Chemistry, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina 27599

[‡]Department of Chemistry, Kwangwoon University, Seoul 139-701, Korea

This review covers publications related to electrochemical sensors that appeared in print during the years 2006 and 2007. The focus of the review is on the development of electrochemical sensing principles including potentiometric and chronopotentiometric sensors, reference electrodes, voltammetric sensors, and electrochemical biosensors.

References were collected by searching electrochemical sensor-related work using *ACS SciFinder* and *ISI Science Citation Index*. In addition, the tables of contents of several analytical journals were reviewed manually to identify relevant articles. Only original and review articles written in English were considered. Patents, book chapters or book series, and conference proceedings or abstracts were not considered for this review. The number of references was limited to 200 and as a result only a fraction of relevant work is covered. Specifically, the review was written to focus on fundamental aspects of electrochemical sensor research emphasizing new chemical concepts and not simply the application of electrochemical sensors. On the basis of these guidelines, several hybrid systems whereby electrochemical detection schemes were combined with a separation technique such as microdialysis, electrophoresis, chromatography, and lab-on-a-chip were not included in this review.

We ask that our readers note that our review is not a comprehensive coverage of the above topics, but rather a snapshot of research conducted over the past two years. Obviously, electrochemical sensor research is still expanding and of interest to a number of disciplines beyond traditional chemistry. As such, it was not possible to cover all manuscripts published. We apologize to the authors of manuscripts that were not included in this review.

POTENTIOMETRIC SENSORS

Reviews

Bakker and Pretsch published a broad review on modern potentiometry describing techniques for improving sensor performance, miniaturizing sensor platforms, and expanding the application of potentiometric sensors for environmental trace analysis and biosensing (98 citations).[1] Likewise, Pretsch provided a general overview on ion-selective electrodes (ISEs) focusing on improving detection limits and understanding non-classical (i.e., non-equilibrium) potentiometry for monitoring clinical relevant polyions such as heparin and protamine (81 citations).[2]

De Marco et al. reviewed ISE methodologies for the analysis of trace metals and anions (e.g., F^-, CN^-, NO_3^-, NO_2^-, and Cl^-) in environmental samples.[3] The review presented a

Correspondence to: Mark H. Schoenfisch.

perspective on the application of ISEs for environmental analyses including real-time remote monitoring of water quality. (147 citations).

Bobacka reviewed the use of conducting polymers as both ion-to-electron transducers (solid contacts) and as sensing membranes in all-solid-state ISEs.[4] The author described use of conducting polymer-based solid-contact ISEs and polymeric ion-selective membranes that have resulted in improvements in sensor performance (e.g., selectivity and stability). The utility of conducting polymers dissolved in the ion-selective membranes was also examined (127 citations). Michalska highlighted the importance of using conducting polymers as solid-contact transducers for fabricating all-solid-state ISEs, and summarized the electroanalytical properties of solid-state potentiometric sensors according to the types of conducting polymers used to construct the solid-contact layer (127 citations). [5]

Bachas and co-workers reviewed several strategies for improving the blood compatibility of potentiometric ion sensors, including the use of more biocompatible polymers (e.g., polyurethane and silicone rubber), surface modifications with hydrophilic copolymers (e.g., poly(ethylene oxide)) and anticoagulants (e.g., heparin), and active membrane release of molecules known to inhibit platelet adhesion (e.g., nitric oxide) (103 citations).[6]

Materials, Ionophores, and Ion Exchangers

Si et al. reported on the synthesis of a thiophene derivative (4-benzeno-15-crown-5 ether)-thiophene-3-methylene-amine monomer self-assembled on a Au(111) surface to promote ordered polymerization and form polymer nanoparticles or clusters with electrochemically controllable sizes.[7] The resulting potentiometric sensor exhibited a selective response towards potassium ions with a detection limit of 40 μM.

Ge et al. demonstrated the use of an ultrathin conducting polymer film as a pH-sensing membrane.[8] Layer-by-layer self-assembly was employed to deposit films on indium-tin oxide (ITO) composed of either one or two bilayers of poly(aniline) (PANI) and poly(acrylic acid) (PAA). Co-deposition of PANI and PAA allowed for the production of a wide distribution of strengths of acidic and basic sites in the film, and thus a wide linear dynamic range from pH 3 to 9.

Denuault and co-workers described a novel pH microelectrode fabricated by electrodepositing mesoporous Pd films onto Pt microdisks and electrochemically charging the film with hydrogen to form the α+β Pd hydride phase.[9] To create the nanostructure, the Pd films were deposited within a molecular template formed via the self-assembly of surfactants. The increased active surface area of the Pd film was necessary to address imperfections (i.e., cracks at the metal-glass interface) in electrodes with sizes below ~50 μm diameter. The performance of the nanostructured $H_{1\text{-}e}$ Pd electrodes was excellent with a Nernstian pH response from pH 2 to 12 that was highly reproducible and stable.

The introduction of new ion recognition chemistries has been one of the main streams of ISE research and remains important today. The Cha and Nam group reported on the synthesis of tripodal thiazole compounds on benzene scaffolds in an effort to enhance cationic selectivity.[10] The benzene ring represents a building block for creating rigid platforms to couple receptors with high selectivity towards cations, anions, and organic molecules. Indeed, ion-selective membrane electrodes doped with the tripodal thiazole derivatives exhibited high sensitivity toward ammonium and silver ions with selectivity patterns depending on the structure of the ionophores employed.

Gupta and co-workers reported the use of mercury-selective membrane electrodes based on the ligand 2-amino-6-purinethiol as a neutral carrier.[11] The authors evaluated the influence

of type and concentration of multiple membrane additives such as plasticizers and lipophilic dopants on the potentiometric sensor performance. The optimized electrode exhibited a Nernstian response towards mercury ions from 70 nM to 0.1 M and a detection limit of 44 nM. The utility of the sensor was demonstrated by determining mercury ion concentrations in environmental (e.g., river, tap, well, and waste water) and biological (e.g., urine, blood, and plasma) samples.

Errachid and co-workers reported the use of dithiomacrocycle (4-phenyl-11-decanoyl-1,7-dithia-azacyclotetradecane-4-sulfide) as a new copper(II) ionophore.[12] With the added neutral carrier, the all-solid-state copper(II)-selective microelectrode exhibited a wide linear response range from 1 μM to 10 mM, a Nernstian slope of 29.5 mV·decade^{-1}, and a detection limit of 562 nM. Sensor response to the copper(II) was not impeded in the presence of common interfering species such as calcium and magnesium.

Ardakani et al. reported on the design of a new oxalate-selective ionophore derived from a metal-naphthoate complex, 2,2′-[1,4-butandiyle bis(nitrilo propylidine)]bis-1-naphthoato copper(II).[13] The electrode exhibited a Nernstian slope of −29.2 mV·decade^{-1}, a wide linear range of 50 nM to 0.1 M, and a desirable selectivity over common interfering species such as perchlorate, acetate, chloride, and nitrate.

Seguí et al. demonstrated the use of linear polyamines as anion carriers for the fabrication of thiocyanate-selective membrane electrodes.[14] Polyamines partially protonated at neutral pH may coordinate anionic species via electrostatic interactions or hydrogen bonds. In this work, the authors evaluated the utility of triamine and tetraamineas thiocyanate-selective ionophores. Optimized electrodes exhibited Nernstian response and detection limits down to 10^{-6} M with adequate selectivity for thiocyanate measurement in biological samples.

Over the past several years, both the Meyerhoff and Malinowska groups have made significant progress in the development of fluoride-selective membrane electrodes. Meyerhoff and co-workers reported on aluminum(III) porphyrin derivatives as potential fluoride ionophores.[15] The aluminum(III) porphyrins possessed a picket fence structure that sterically hindered interactions with other porphyrins (i.e., preventing dimerization), resulting in the elimination of undesirable super-Nernstian behavior.

Previously reported fluoride sensors based on metalloporphyrins exhibited super-Nernstian characteristics, slow response, long recovery times, and irreproducible response toward fluoride. Malinowska and co-workers reported the use of aluminum(III)- and zirconium(IV)-tetraphenylporphyrin derivatives as fluoride ionophores.[16] The effects of phenyl-ring substituents and the identity of metal centers on the complexation affinity of the metalloporphyrins with various anions were investigated. Resulting fluoride-selective sensors showed improved performance compared to previously reported sensors with respect to Nernstian response, reproducibility, and lifetime (up to 7 months).

Direct potentiometry and potentiometric titrations for the determination of organic medicinal drugs (i.e., pharmacologically relevant ionic species) are well known applications of ISEs.[17] To detect diclofenac (DCF), a potent non-steroidal anti-inflammatory agent, in pharmaceutical samples, Santini et al. employed a Hg|$Hg_2(DCF)_2$|graphite pellet.[18] The sensor showed excellent response to DCF with a sensitivity of 58 mV·decade^{-1} over 50 μM to 10 mM DCF at pH 6.5 to 9.0 and a detection limit of 32 μM.

A molecular imprinting technique was employed by D'Agostino and co-workers to develop a potentiometric sensor for atrazine, an s-triazine-ring herbicide.[19] The imprinted polymer membrane was prepared by polymerizing methacrylic acid and ethylene glycol dimethacrylate in the presence of atrazine. The atrazine was then removed by washing with

methanol/acetic acid (4:1, v:v). The resulting sensor provided a good analytical response towards atrazine with a sensitivity of 25 mV·decade^{-1} over 30 μM to 1 mM, a response time of <10 sec, and a sensor lifetime of >2 months.

Yin et al. examined the enantioselectivity of optically active poly(aniline) (PAn) films to recognize D- and L-phenylalanine (Phe).[20] To enable enantioselective recognition ability on the potentiometric polymer membrane, the authors prepared two types of PAn films containing (1*S*)-(+)- or (1*R*)-(−)-10-camphorsulfonic acid (CSA) known as (+) PAn and (−) PAn, respectively. While the slope of the (−) PAn-based sensor for L-Phe was approximately 59 mV·decade^{-1}, the slope for D-Phe was sub-Nernstian (35 mV·decade^{-1}).

Sol-gel chemistry was employed by the Javanbakht group to produce an dipyridyl-functionalized nanoporous silica gel. The silica film was used to fabricate copper-selective carbon paste electrodes.[21] The dipyridyl group modification on the porous silica film served as the ionophore for copper(II) binding, enabling selective binding. Furthermore, the immobilization of the functional dipyridyl group on the silica backbone resulted in a significant improvement in sensor lifetime (~9 months).

Nam, Brown, and co-workers described a novel platform for fabricating all-solid-state ion-selective electrodes using a nanoporous glass capillary.[22] The electrode was fabricated by sealing a conically-etched Pt wire (d = 25μm, radius of etched tip <10 nm) in a glass capillary. A Pt disk was then exposed by polishing and etching the glass to form a conical pore with radius <500 nm and depth <30 μm. Silver was electrodeposited on the platinum and chloridated to form a Ag/AgCl layer within the pore. The authors demonstrated the analytical performance of the resulting glass-nanopore electrode by preparing a pH sensor via deposition of the IrO_2 layer. The sensor exhibited a 80 mV·pH^{-1} potentiometric response from pH 3 – 12.

Detection Limits and Selectivity Coefficients

Improved selectivity and sensitivity have revolutionized the field of ionophore-based ISEs. Since the initial fabrication of Pb^{2+}-selective membrane electrodes capable of trace ion measurements down to 10^{-11} M, many research groups have applied similar strategies for measuring other ions. The recent trend of developing ISEs with lower detection limits has focused on a solid contact, which consists of a solution-free internal layer located between the sensing membrane and the metal electrode.

The groups of Bakker and Pretsch continue to develop unique strategies for lowering the detection limits of polymeric ion-selective membrane electrodes. Bakker's group recently compared several strategies known to influence detection limits, while considering practical applicability.[23] Radu et al. reported guidelines for lowering the detection limit via internal filling solution composition, aqueous diffusion layer thickness, the diffusion coefficient, and the amount of ion exchanger in the membrane.[24] Subfemtomole detection limits were demonstrated using a microelectrode in confined sample volumes from 30 – 50 μL. Malon et al. reported a detection limit of 100 pM for Ca^{2+}, Pb^{2+}, and Ag^{+} in sample volumes as low as 3 μL.[25] In a subsequent study, a Ag^{+}-selective membrane electrode with a poly(3-octylthiophene)-based conducting layer was evaluated in 50 μL volumes.[26] The sensor's response was reproducible with a detection limit of 4 nM.

Any water existing between the ion-selective membrane and the inner solid contact often leads to a less than optimal sensor detection limit. Sutter and Pretsch compared several inner contacts including polypyrrole formed by electropolymerization in the presence of KCl and poly(3-octylthiophene).[27] Poly(vinyl chloride) (PVC) and polyurethane were also evaluated to determine the most promising polymer matrix for solid-contact ISEs. The best results

were obtained from PVC-based membrane electrodes with a poly(3-octylthiophene) solid contact.

Szigeti et al. evaluated the sensitivity, selectivity, and complex formation constants of several Ag^+ ionophores in solvent polymeric membranes.[28] Bridged thiacalixarenes exhibited the most promising detection limits for potentiometric and optical Ag^+ sensors, 30 pM and 20 pM, respectively.

New Concepts, Mechanisms, and Characterization

Wanichacheva et al. reported the use of a self-assembled monolayer as a potentiometric sensing surface.[29] A triazabicyclic lithium-selective ionophore was anchored to a hexadecanethiol chain and subsequently immobilized via self assembly on a gold surface. The cation recognition properties were evaluated with cyclic voltammetry, impedance spectroscopy, and potentiometry.

A method for eliminating the calibration process for ion-selective membrane electrodes was introduced by Malon and co-workers using a technique known as backside-calibration potentiometry.[30] In this technique, the magnitude of the observed potential is not important. Furthermore, the technique does not rely on the Nernst equation. The sample side of the membrane is never altered for calibration purposes. Rather, the inner solution composition is varied until the concentration gradient across the ion-selective membrane is reduced to zero, similar to zeroing a Wheatstone bridge. Thus, the disappearance of signal drift may be used to determine the sample composition. To extend the utility of backside-calibration potentiometry, several parameters affecting sensitivity and working range were explored including the primary ion concentration ratio, interfering ion concentration, and plasticizer type.[31]

Lingenfelter et al. described a new theoretical approach for determining selectivity coefficients of ion-selective membrane electrodes using the Nernst-Planck-Poisson (NPP) equation.[32] Previous selectivity prediction methods have been restricted by certain assumptions (e.g., steady state phase boundary and electroneutrality). In contrast to the steady-state phase boundary approach, the NPP model enabled changes in potential response and variability in selectivity to be predicted with variations in concentration ratio and time.

Michalska et al. offered a new approach for evaluating ion-depth profiles across ion-selective membranes via laser ablation inductively coupled plasma mass spectrometry.[33] The technique provided insight into the transmembrane properties of ion fluxes without compromising the membrane composition. The authors revealed that the presence and distribution of ionic fluxes (i.e., primary and interfering ions) across the membrane were strongly governed by the composition of the solution to which both sides of the membrane were exposed during preconditioning, and the plasticizer included in the membrane formulation.

del Valle and co-workers demonstrated the use of electrochemical impedance spectroscopy (EIS) to elucidate the super-Nernstian behavior between tetronasin, an antibiotic ionophore, and calcium ions.[34] The EIS data indicated that tetronasin exhibited extremely fast ion exchange characteristics. The resulting super-Nernstian response was in agreement with previous interpretations using both potentiometric and impedance analysis.

For applications requiring the use of microfluidic devices where sample volume and analyte concentrations are small, sensor capacitance may be a performance-limiting factor. Majda and co-workers introduced an electrochemical time-of-flight method based on a dual-microelectrode system consisting of generator and sensor electrodes to determine the

capacitance of solid-state potentiometric sensors.[35] The electrochemical time-of-flight technique generated potential-time (*E*–*t*) transients that were used to characterize the response time and capacitance of silver/silver chloride and iridium oxide microsensors.

The replacement of aqueous inner solutions with a solid contact inner membrane has increased the potential stability of ISEs. Recent work has focused on improving detection limits of such electrodes. Bakker and co-workers described polymeric ion-selective membrane electrodes modified with poly(3-octylthiophene) as a solid inner contact via solvent casting on gold-sputtered copper electrodes.[36] Five different ISEs for Ag^+, Pb^{2+}, Ca^{2+}, K^+, and I^- were studied, showing good detection limits and excellent reproducibility.

A new type of solid contact was demonstrated by Lindfors et al. using plasticized poly(vinyl chloride) (PVC) membranes containing PANI nanoparticles (d = 8 nm).[37] In contrast to electrochemically deposited PANI films, the PANI nanoparticle-based solid contacts exhibited exceptionally good pH stability. The conducting layer remained stable at pH 7.5 for at least a month without any degradation in conductivity. Furthermore, the use of such a polymeric solid contact allowed for improved reproducibility and good mechanical strength between the inner and outer membranes.

Bühlmann and co-workers reported the use of three-dimensionally ordered macroporous (3DOM) carbon as an inner ion-to-electron transduction layer for fabricating a new class of solid contact ISEs.[38] The 3DOM carbon proved to be a promising candidate as a solid contact with high ionic and electrical conductivity due to well-defined interconnected pore and wall structure.

Perrot and co-workers employed as alternating current (ac)-electrogravimetry to better understand all-solid-state potassium (K^+) ISEs using polypyrrole layers as the inner solid contacts.[39] The study of ion and solvent motions at the membrane/electrolyte interface via ac-electrogravimetry revealed that K^+ ions enter the membrane. Such behavior confirmed that PVC K^+-selective membranes enhance cation insertion as controls (polypyrrole without the PVC sensing membrane) were anion selective. These results may explain both poorer sensitivity at lower analyte concentrations and limited sensor lifetimes.

CHRONOPOTENTIOMETRIC SENSORS

Although significant advances have been made in the area of ion-selective electrodes over the past century, the field of direct potentiometry has faced numerous challenges. Direct potentiometric methods have inherently lower sensitivities as they depend on the logarithmic relationship between the observed potential and the sample activity. Additionally, such methods require both a reliable reference electrode with a liquid junction and an electrolyte reservoir of constant composition. These limitations have hindered the widespread use of ISEs in clinical monitoring, in vivo measurements and remote environmental analysis.

The Bakker group recently reported the use of pulsed chronopotentiometrically controlled ion-selective electrodes, termed pulstrodes, to overcome common restrictions of classical zero current potentiometry. Such measurements are based on a three-step pulse experiment. An applied current pulse first extracts ions from the sample into the ion-selective membrane, leading to concentrated ion fluxes inside the membrane. In the second step, the potential is measured under traditional zero current conditions. Finally, a potential pulse is applied to drive previously extracted ions back into the sample solution. Ion-selective membranes interrogated with this triple-pulse technique eliminate *iR* drop, thus resulting in excellent sensitivity. Recently, the authors reported on a calcium pulstrode with a 10-fold enhanced response when compared to traditional calcium ISEs.[40]

Gemene et al. described a chloride sensor measured in the pulsed chronopotentiometric mode.[41] The chloride pulstrode exhibited 100-fold improved selectivity over perchlorate and salicylate, common interfering species for classical chloride ISEs. The improved selectivity was attributed to the robust kinetic discrimination over the interfering ions. Perera and Shvarev demonstrated a potassium pulstrode with a 1000-fold increased selectivity over magnesium compared to traditional ISEs.[42] The pulstrodes did not require counterbalancing of the transmembrane ionic fluxes to achieve unbiased thermodynamic selectivity, allowing their use under asymmetric conditions. As such, the fluxes of the inner filling ions across the membrane neither influenced sensor response nor selectivity.

Bakker and co-workers introduced a new method for tuning the ion selectivity of pulstrodes by modifying the sensing membrane with polyelectrolyte multilayers.[43] The positively or negatively charged multilayers drastically altered the kinetics of ion transport to the membrane, thereby providing an effective means of controlling both ion permeability and ion selectivity.

Perera et al. extended the utility of pulse chronopotentiometry to solid contact-based ion sensors by developing a solid-state protamine sensor with a poly(3,4-ethylendioxythiophene)-poly(styrene sulfonate) layer as a solid contact.[44] The resulting protamine sensors exhibited an improved detection limit of 0.03 $mg \cdot L^{-1}$.

REFERENCE ELECTRODES

The reference electrode is an essential component of electroanalytical systems. Many types of conventional reference electrodes have been developed previously. Nevertheless, the miniaturization of sensors now warrants increasingly smaller and less complex reference electrodes. Liao and Chou described the fabrication of planar-type reference electrodes via straightforward screen-printing methods.[45] The reference electrode employed agar gel as a solid inner electrolyte. Chloroprene rubber was used as a liquid junction and insulator. The planar reference electrode response was insensitive to most physiologically relevant ionic species including Na^+, K^+, Ca^{2+}, NH_4^+, and Cl^- up to 0.3 M over pH 2 to 10.

Shim and co-workers developed an all-solid-state reference electrode by coating a AgCl surface with a silicone rubber film containing KCl salt and Nafion, and a polyurethane membrane containing a proton ionophore, plasticizer, and lipophilic additive.[46] The reference electrode exhibited improved long-term stability for both potentiometric and voltammetric measurements.

Kakiuchi et al. utilized a hydrophobic ionic liquid to fabricate a Ag/AgCl reference electrode. The electrode consisted of a Ag/AgCl wire coated with a AgCl-saturated ionic liquid and 1-methy-3-octylimidazolium bis(trifluoromethylsulfonyl)imide as an internal electrolyte.[47] Using a gelled ionic liquid salt bridge, this reference electrode demonstrated stable potentials in solutions up to 2 M KCl.

A miniaturized needle-type Ag/AgI reference electrode was reported by Hashimoto et al.[48] As determined by the solubility of AgI, the internal iodide concentration was maintained at a constant level. The active junction, made with poly(vinyl alcohol) and starch, blocked both the external iodide and reduced the influence of interfering species. The resulting reference electrode was successfully employed to determine pH values in the presence of common interfering species with a precision of ±0.01 pH unit.

The groups of Kim and Chung demonstrated the use of a polyelectrolyte as a salt bridge for fabricating miniaturized reference electrodes.[49] The polyelectrolyte liquid junction was formed via photopolymerization of diallyldimethylammonium chloride in the presence of

saturated KCl. The reference electrode maintained a stable, reproducible potential for periods greater than 30 h, with no significant interferences. The Chung group also reported the fabrication of a solid-state reference electrode for voltammetric measurements using electrodeposited nanoporous platinum.[50] The poly-1,3-phenylendiamine modified platinum oxide effectively prevented interferences from redox couples. Additionally, the large pH response of the nanoporous platinum oxide electrode enabled stable potentials in buffer solutions.

Bard and co-workers described the development of a new quasi-reference electrode (QRE) for voltammetry in both nonaqueous and aqueous solutions.[51] The QRE was easily fabricated by electrodepositing Pt with partially oxidized polypyrrole. The solid-state junction-free QRE showed good stability over several days. The elimination of the liquid junction addressed salt leaching contamination.

VOLTAMMETRIC SENSORS

Reviews

The trend towards miniaturization of sensor platforms has led to an emphasis on the micro- and nano-fabrication of electrochemical sensors. As a result, the development of microsensors has been the focus of a large number of recent electrochemical sensor publications due to a number of factors including the potential for high spatial resolution.

Nagy and Nagy presented a thorough review of the development and progression of microelectrode research and highlighted examples of recently reported microelectrodes (107 citations).[52] Brief discussions of the fabrication and applications of potentiometric and voltammetric microsensors were included as well. Ordeig et al. authored a review detailing the theory and applications of microdisk electrode arrays, making a strong case for their use over single microelectrodes despite the increase in ohmic drop observed at arrays (185 citations).[53] The authors stressed that microelectrode arrays maintain key advantageous properties of single microelectrodes such as low capacitive currents, and are thus more suitable for a majority of electroanalytical applications due to increased sensitivity and improved signal-to-noise. Much of the review focused on modifying arrays with mercury, polymer and inorganic films, boron-doped diamond, and enzymatic biosensors.

The use of nanostructures including nanotubes and nanoparticles continue to be a major focus of electrochemical sensor fabrication due to their unique chemical and physical properties, such as high surface to volume ratio. Riu et al. detailed the use of sensors based on nanostructures having at least one dimension of 100 nm or less (121 citations).[54] The review covered many classes of nanostructures and described the fabrication, physicochemical properties, and experimental advantages of each material. In addition, the authors detailed the use of such sensors for environmental analysis. Luo et al. reviewed the use of nanoparticles in electrochemical sensors with a focus on catalysis, electron transfer, immobilization, labeling of biomolecules, and their use as reactants (62 citations).[55] The authors pointed out several examples of each nanoparticle application and highlighted the advantages of their use.

Recent work related to implantable electrochemical sensors has focused on improving the biocompatibility and long-term stability of such sensors. Frost and Meyerhoff described both the challenges associated with implantable chemical sensors and several strategies for improving the response of intravascular and subcutaneous sensors (66 citations).[56] The authors focused on sensors that release nitric oxide (NO) to reduce biofouling due to platelet adhesion and the inflammatory response. Shin and Schoenfisch highlighted the use of NO to improve the biocompatibility of several in vivo sensors (47 citations).[57] The authors

provided details regarding common NO release coatings and described the coupling of NO release chemistry with successful sensor fabrication.

Wang reviewed recent advances in the development of electrochemical sensors used to detect explosives under challenging conditions such as gas-phase samples and marine environments (46 citations).[58] The review covered the electrochemistry of organic explosives including polynitro aromatic and peroxide compounds, and provided examples of sensor platforms. Microchip-based explosives sensors were highlighted due to their small size, low power consumption, and ease of integration with sample processing systems, making them ideal for use in the field.

Renedo et al. reviewed recent developments and applications of screen-printed electrodes including unmodified, film-coated, enzyme-modified, and immunosensors (218 citations).[59] The low cost and ease of fabrication related to printable materials has led to the increased study of screen-printed electrodes as evidenced by the sheer volume of recent publications.

Barek et al. reviewed recent progress on the use of non-traditional electrode materials such as solid amalgam, carbon paste, and boron-doped diamond for electrochemical sensor fabrication (155 citations).[60] The authors discussed the application of detecting environmentally relevant organic molecules using solid amalgam electrodes, a more environmentally benign alternative to mercury electrodes.

Esteban and co-workers examined the state of chemometrics research as it relates to the analysis of voltammetric data (65 citations).[61] Specifically, the authors focused on multivariate-analysis techniques for multianalyte calibration and resolving dynamic multicomponent systems.

Theory, Modeling, and Analysis

Studies continue to be focused on electrochemical theory at the surface of the electrode. Chang and Park demonstrated a model based on equivalent circuits that describe both the faradaic and non-faradaic currents at the interface of the electrode and electrolyte during a potential step experiment.[62] The authors verified their theory using electrochemical impedance spectroscopy, noting that their results may impact the general description of the electrode/electrolyte interface. Wightman's group presented a theoretical description of the current at an inlaid microelectrode, verifying their results using carbon fiber microelectrodes.[63] The authors reported that although exact expressions may be found for inlaid microelectrodes with circular and elliptical shapes, approximate expressions are still obtainable for electrodes of any connected shape with finite area. Anastassiou et al. presented a method for determining the physical parameters of heterogeneous electrochemical reaction-diffusion systems using large-amplitude ac voltammetry.[64] Contributions to the signal from capacitance were minimized by using ac voltammetry in combination with signal processing based on the Hilbert transform. The physical parameters for two well-characterized electrochemical systems were determined in a fraction of the time needed to calculate previously reported literature values. A method for determining both thermodynamic and kinetic parameters of an electrochemical system from a single staircase voltammetric experiment was reported by Chang and Park.[65] The authors utilized a new method of Fourier transform electrochemical impedance spectroscopic (FTEIS) analysis to determine an equivalent circuit. From this circuit, several parameters were extracted including the diffusion coefficient, electron-transfer rate constant, double layer capacitance, and number of electrons transferred. This method represents a significant improvement over previous methods used to determine such parameters in terms of both simplicity and efficiency.

Sun and Mirkin carefully evaluated previous reports concerning extraordinarily high rate constants at nanoelectrodes.[66] The connection between the rate of electron-transfer reactions and the size of the electrode was investigated using voltammetry, scanning electron microscopy, and scanning electrochemical microscopy. These studies revealed that the difference in electron transfer rates between nano-scale and larger electrodes was slight, and thus previous reports were flawed due to the lack of available techniques to accurately characterize nanoelectrode geometry.

Morf and co-workers presented a thorough theoretical analysis of steady-state and time-dependent amperometric currents at uniform microelectrode arrays (only cathodes or anodes) and alternating cathodes and anodes (interdigitated arrays).[67] Accurate behavior predictions of such microelectrode arrays may allow for improved designs and a better understanding of how array geometry influences response characteristics.

Bieniasz and Rabitz examined the use of high dimensional model representation (HDMR) as a method for the analysis of complex cyclic voltammetry data.[68] The authors acknowledged that the most challenging aspect of using HDMR is the creation of solution maps. However, once generated, such maps would greatly increase experimental CV data analysis.

Compton's group reported a theory describing voltammetric signals during the stripping step of cathodic and anodic stripping voltammetry.[69] The authors proposed multiple mathematical models based on the thickness of the deposition layer on a solid electrode. Furthermore, they noted that a general theory of stripping voltammetry may allow for the collection of mechanistic data not previously available.

Stripping Voltammetry

The inherent toxicity of mercury electrodes has led to a focus in developing alternative electrode materials for use in stripping voltammetry. Hocevar et al. reported the use of an antimony film electrode for trace Cd(II) and Pb(II) analysis, demonstrating performance similar to mercury and bismuth-based electrodes.[70] Compared to their bismuth-film counterparts, the antimony-film electrodes exhibited the additional advantage of improved performance in acidic environments (pH < 2). Kefala and Economou evaluated the use of Nafion-coated bismuth film electrodes for the analysis of cadmium, lead, and zinc using anodic stripping voltammetry.[71] The authors noted that the Nafion coating allowed for the reliable determination of Cd(II), Pb(II) and Zn(II) in the presence of surfactants, down to detection limits of approximately 18, 10, and 92 nM respectively. Hutton et al. employed a bismuth film electrode (BiFE) for trace analysis of tin using anodic stripping voltammetry. The detection limit of the BiFE for tin was 2.2 nM.[72] Compared to mercury film and bare glassy carbon electrodes, the BiFE electrodes exhibited improved performance, especially in to the presence of interferences such as catechol. Prior and co-workers demonstrated the use of BiFEs for the trace determination of copper via anodic stripping voltammetry.[73] Previous attempts to use BiFEs for copper determination proved unsuccessful due to the overlap of Cu and Bi stripping peaks. The authors utilized gallium to form a Ga-Cu compound, diminishing competition for the electrode surface between Cu and Bi and allowing for detection of Cu down to 1.4 $\mu g\ L^{-1}$. Berduque et al. fabricated gold and platinum microelectrode arrays and demonstrated their use in the mercury-free stripping voltammetric determination of copper ions.[74] The authors tested multiple electrode dimensions. The Cu(II) content of a soil sample was determined with results comparable to that of atomic spectroscopy.

van den Berg combined 2,3-dihydroxynaphthalene (DHN) and cathodic stripping voltammetry to determine the complexation of iron in seawater samples.[75] When compared to methods using ligands such as salicylaldoxime, this method demonstrated greater

sensitivity to iron, improved response time, and a better limit of detection. The simultaneous determination of copper and mercury in seawater by anodic stripping voltammetry using gold microwires was reported by Salaün and van den Berg.[76] The negative influence of halide anions on the stability of the mercury signal was attenuated by including an anion desorption step before each scan. The authors reported detection limits of 6 and 25 pM for mercury and copper, respectively.

Xiao and co-workers reported of the use of an ultramicroband array electrode to measure trace mercury in soils by anodic stripping voltammetry.[77] The array was composed of thin bands of gold-plated iridium electrodes (0.2 by 6.0 μm). Under ideal conditions, the array was able to detect mercury concentrations as low as 2.5 nM in dilute acid. To detect mercury in soil samples, the authors performed an acid extraction. Levels of mercury as low as 5 μM were measured.

Baś described a method for the determination of cadmium at the surface of a mercury film silver based electrode via catalytic adsorptive stripping voltammetry.[78] The advantages of the resulting electrode over a traditional hanging drop mercury elecrode included both improved mechanical resistance and increased surface area. The sensor exhibited a detection limit of 0.19 nM Cd.

Yong et al. described a novel method of pretreating blood samples for subsequent chromium (Cr) analysis by catalytic adsorptive stripping voltammetry (CAdSV).[79] An advanced oxidation process utilizing hydrogen peroxide and ultraviolet light was effective in releasing Cr from its biological complexes in blood. Analysis of pretreated blood samples yielded concentrations of chromium that were identical to those determined by atomic absorption spectroscopy.

Performing stripping voltammetry in small volumes is difficult due to the impracticality of traditional convection. Anderson and Fritsch report on a number of factors affecting redox magnetohydrodynamic-induced (MHD) convection (including Fe(III) concentration and magnetic field strength) in small volumes.[80] Adequate MHD convection was produced during linear sweep anodic stripping voltammetric analysis of Cd(II) in volumes as small as 100 μL to attain limits of detection of 69 nM.

Wang's group reported the use of a mercury film electrode for the trace determination of beryllium via adsorptive stripping voltammetry.[81] The authors utilized a beryllium–arsenazo-I complex to increase sensitivity to beryllium, allowing for both detection down to 0.25 μg L^{-1} and reuse of the electrode after a short cleaning step.

Materials

The use of electrochemical sensors for enantiomeric recognition has generated continued interest in the field. The ability to determine and control enantiomeric purity is especially important for the development and production of pharmaceuticals. Chun and coworkers reported on an enantiomeric selective sensor for alkyl amines utilizing a nitroazophenolic crown ether having an oxidation potential that varies with analyte structure.[82] The resulting oxidation potential difference between pure *R* and *S* enantiomers was 35 mV, and varied linearly with the ratio of *R* to *S*. El-Hady et al. reported the development of an enantiomeric selective sensor for the determination of the immunosupressor drug methotrexate.[83] The authors utilized hydroxypropyl β-cyclodextrin to adsorb and quantify methotrexate enantiomers via adsorptive stripping voltammetry with an enantioresolution of 2.96.

Ivandini and co-workers reported on a boron-doped diamond electrode modified with iridium ions capable of catalyzing the oxidation and detection of arsenic via cyclic

voltammetry.[84] The sensor surface was characterized using several microscopic and spectroscopic methods. With a limit of detection of 20 nM As(III), the performance of the sensor represents an attractive alternative to more conventional arsenic measurement strategies (e.g., anodic stripping voltammetry) due to high sensitivity and stability.

Donner et al. reported the fabrication of robust, optically transparent thin-film carbon electrodes by pyrolyzing dilute solutions of positive photoresist on quartz surfaces.[85] The authors described both the optical and electrical properties of the electrodes. In addition, preliminary results from the first reported example of a combination of single molecule spectroscopy and electrochemistry were provided.

Chicharro et al. explored the use of tape casting to fabricate carbon electrodes more easily than traditional carbon paste electrodes (CPE).[86] The method involved the use of non-aqueous tape casting additives (e.g., binders and plasticizers) instead of mineral oil. These additives function to form strong, self-standing carbon electrodes (SSCE). The authors compared the performance of SSCEs and CPEs for measuring norepinepherine via amperometry. The resulting sensitivity of SSCEs was one order of magnitude greater than that of CPEs. In a related report, Maleki et al. described the use of a ionic liquid, n-octylpyridinum hexafluorophosphate (OPFP), as a binder for graphite.[87] The authors demonstrated that the resulting electrode exhibited a number of improvements over CPEs including a lower required potential for sensing several biomolecules (e.g., NADH, ascorbic acid, and dopamine), low background, high strength, and reduced NADH fouling.

Kahn and co-workers described the synthesis of an aminoferrocene-derivative film to sensitize a gold electrode to various chemical warfare agents.[88] Simulants for sulfur mustard and tabun shifted the anodic redox potential for ferrocene, as monitored by cyclic voltammetry and differential pulse voltammetry.

Salimi et al. described the fabrication of a carbon ceramic electrode (CCE) modified with nickel powder and potassium octacyanomolybdate(IV) via the sol-gel process.[89] The modified electrode allowed for the detection of insulin over a linear range of 100–400 pM with a detection limit of 40 pM.

Yang and co-workers reported the development of a glassy carbon electrode modified with poly(brilliant cresyl blue) (PBCB) via electropolymerization.[90] The resulting sensor was used to measure nitrite in food products. By reducing the potential required to measure nitrite, the PBCB modification increased the selectivity over common ionic interferences and cholesterol. The response of the sensor was linear from 0.9 to 15.0 μM with a detection limit of 0.1 μmol.

Increasing the lifetime and biocompatibility of in vivo sensors by the release of nitric oxide (NO), an inhibitor of platelet adhesion and activation, from the sensor surface has led to the development of several NO-releasing sensor membranes. Meyerhoff's group has worked to address the finite NO storage capacity of most polymer coatings by generating NO in situ via copper catalyzed decomposition of endogenous NO transporters, *S*-nitrosothiols.[91] Using such coatings, intravascular oxygen sensors were fabricated and carefully evaluated in vivo in porcine arteries. Results were promising, indicating improved long-term sensor response and reduced thrombosis at the sensor surface.

Ulyanova and co-workers described the development of a molecularly-imprinted polymer composed of electropolymerized methylene green. Theophylline sensors were fabricated using the modified polymers.[92] The measurement of theophylline, an asthma drug, was reported down to 3 nM with good selectivity over caffeine, a major interferent. The authors

noted that poly(methylene green) shows promise as a robust sensor imprinting matrix for a variety of analytes.

Likewise, an acrylic molecularly-imprinted photoresist (MIPhs) was employed by Huang and co-workers to fabricate a multi-array electrochemical sensor with 20 μm dimensions by standard photolithographic methods.[93] The utility of the resulting sensor array was demonstrated by detecting albuterol down to a detection limit of 1 μM. Clearly, MIPhs are promising materials for the construction of highly selective, multi-array sensors.

Carrington and co-workers reported the fabrication of a sensitive chromium sensor via the electrodeposition of a pyridine-modified sol-gel film on a glassy carbon electrode.[94] The sol-gel membrane facilitated the pre-concentration of anionic chromium and allowed for its detection via square wave voltammetry down to 4.6 ppb, with minimal interference from Cr(III).

Nanomaterials

Su et al. reported the synthesis of multiwalled carbon nanotube (MWCNT) films as sensor membranes by their controlled adsorption onto gold electrodes modified with a self assembled monolayer (SAM).[95] By adjusting the MWCNT adsorption time, the electrode dimensions were carefully controlled. A decrease in capacitance at the interface of the electrode was noted compared to previously reported MWCNT electrodes. Yogeswaran and co-workers developed a biomolecule sensitive film electrode based on nano platinum, nano gold, Nafion, and MWCNT composites.[96] The simultaneous determination of ascorbic acid, uric acid, and epinephrine was demonstrated using cyclic voltammetry and differential pulse voltammetry with excellent reproducibility and long-term electrode stability. Compton's group reported on the fabrication of multianalyte sensors using metal particle-modified (Pd, Au, and Pt) glassy carbon microspheres bound by a film of MWCNTs.[97] The resulting sensor response was similar to that of macroelectrodes of the same materials using only a fraction of the bulk metals. The electrode system behaved as a macroelectrode of each different metal simultaneously, thus indicating the potential for combinatorial electrochemistry. Zhang and co-workers presented a method of fabricating gas sensors using polyaniline-modified single walled carbon nanotubes (PANI-SWNT).[98] The use of PANI-SWNTs as a sensitive ammonia gas sensor was demonstrated with a limit of detection of 50 ppb. The resulting materials may facilitate the development of sensor arrays of individually addressable components for the detection of complex mixtures of analytes.

Wang's group described the electrodeposition of ruthenium oxide (RuOx) onto CNT-modified carbon electrodes to facilitate the electrocatalytic measurement of insulin.[99] The enhanced electrocatalytic activity towards insulin was attributed to a lowered oxidation potential and enhanced sensor response. The RuOx/CNT-modified carbon electrodes exhibited a wide linear dynamic range of 10–800 nM and limit of detection of 1 nM.

Li and Lin fabricated a polypyrrole-modified glassy carbon electrode with electrodeposited gold nanoclusters for the simultaneous determination of uric acid and epinephrine in the prescence of ascorbic acid.[100] The authors reported a synergistic effect between the polypyrrole film and gold nanoclusters that allowed for the determination of uric acid and epinephrine down to 12 nM and 30 nM, respectively.

Hrapovic et al. electrodeposited platinum nanoparticles on boron-doped diamond electrodes to enable the sensitive detection of As(III) in water using linear sweep voltammetry.[101] The resulting electrode was able to measure As(III) over a wide-linear range up to 100 ppb and a detection limit of 0.5 ppb with no interference from copper or chloride ions.

Sun and co-workers synthesized single-walled carbon nanotubes filled with ferrocene, termed ferrocene peapods, to combine the favorable features of each component with respect to electron transfer.[102] The use of the ferrocene peapod as a hydrogen peroxide (H_2O_2) sensor was demonstrated with good stability and reproducibility. Although the detection limit of the resulting sensor did not surpass that of other recently reported H_2O_2 sensors, the concept of tailoring the electrochemical properties of carbon nanotubes by using small molecules represented an exciting endeavor, warranting further investigation as potential sensor components.

Forzani et al. combined the advantages of amperometry and coulometry by fabricating electrode arrays connected by a polyanaline bridge that allowed both methods to be performed simultaneously.[103] Using the hybrid system, small changes in dopamine concentration (100 nM) were measured in the presence of high concentrations (1 mM) of ascorbic acid.

Analytes of Interest

The detection of nitroaromatic compounds continues to generate enormous interest due to their relevance in national security and environmental monitoring. Electrochemical sensors are especially attractive owing to their simplicity, low cost, and portability compared to other detection strategies. Hrapovic et al. reported on the detection of 2,4,6-trinitrotoluene (TNT) down to 1 ppb via adsorptive stripping voltammetry.[104] Copper (Cu) nanoparticles and carbon nanotubes were combined to form a composite electrode capable of detecting a wide array of nitroaromatic compounds. Using mesoporous silicon dioxide-modified glassy carbon electrodes, Zhang and co-workers were able to measure 2,4,6-trinitrotoluene down to the 0.5 ppb level via cathodic voltammetry.[105] The SiO_2 proved to be an excellent adsorbent for nitroaromatic compounds. As a result, the preconcentration of TNT on the electrode surface was quick. Indeed, the voltammetric detection was demonstrated in less than 15 sec. Chen et al. utilized the characteristic substituent-dependent potential shifts of various nitroaromatic compounds as a detection strategy.[106] A simple screen-printed anodized carbon electrode was used in a three-electrode configuration to provide a method for identifying and detecting chloramphenicol, parathion, and TNT by their unique potential shifts using square wave voltammetry.

The electrochemical measurement of biologically-relevant thiols has been the subject of recent interest because of their prevalence as indicators for a number of diseases. Pacsial-Ong and co-workers reported the selective detection of glutathione (GSH) via the formation of thiol adducts with redox-active indicators such as fluorine black.[107] The GSH formed bisthiol adducts were detectable at more anodic peak potentials than cysteine and homocysteine aminothiols capable of forming only monothiol-indicator adducts. As such, GSH selectivity was achieved. Chen et al. reported the detection of GSH over a linear range of 0.1 to 2.8 mM without interference from uric and ascorbic acids using an acetylene black-packed powder microelectrode (AB-PME).[108] The oxidation potential of GSH was shifted to a more negative value, allowing for its discrimination from interfering species. While the electrochemical oxidation of GSH is typically irreversible, the AB-PME increased the reversibility of the system. Zhou and co-workers reported on the fabrication of a *l*-cysteine sensor using ordered mesoporous carbon-modified glassy carbon (OMC-GC) electrodes.[109] Such sensors exhibited responses over a wide-linear range (18–2500 μM) with detection limits of ~2.0 nM. The disappearance of one of the three anodic peaks during the oxidation of *l*-cysteine was noted above pH 5.00, a phenomenon not previously encountered.

Lyon and Stevenson described a novel method for measuring hydrogen peroxide (H_2O_2) at a microelectrode via square wave voltammetry.[110] Amplex red, a redox-mediator for hydrogen peroxide that is activated in the presence of horseradish peroxidase, allowed for

the quantification of H_2O_2 down to 8 pM. In contrast, Miah and Ohsaka reported on a sensor capable of measuring very high concentrations of H_2O_2 using gold electrodes.[111] The controlled electrochemical desorption iodide on the electrode allowed for the reduction of hydrogen peroxide at the freshly exposed gold surface, circumventing the oxidative degradation normally associated with measuring elevated concentrations of H_2O_2 at bare metal electrodes and the adsorption of impurities at the electrode surface.

Schoenfisch's group reported on the fabrication of a planar ultramicroelectrode NO sensor capable of measuring NO release from a surface with high spatial resolution and without any deleterious effects from analyte trapping.[112] A silicone-rubber gas permeable membrane was used to modify platinum ultramicroelectrodes. The sensor performance was characterized as having response times on the order of a few seconds and a detection limit of 5 nM, indicating suitability for biological measurements. Griveau et al. described an electrochemical sensor for measuring both exogenous and induced endogenous NO in a mouse tumor tissue.[113] The authors noted that their sensor would be well adapted for studying the in vivo effects of anti-cancer drugs on NO pathways. Nickel hexacyanoferrate-modified platinum electrodes were fabricated by Krylov and Lisdat for use as NO sensors.[114] The modification reduced the potential needed for the NO measurements to 250 mV, resulting in a response to NO more than one order of magnitude greater than common interferences (e.g., hydrogen peroxide, nitrite, nitrate, dopamine, and ascorbic acid) without the use of a barrier membrane. Lee and Kim reported the development of a dual planar amperometric microsensor capable of measuring both NO and carbon monoxide (CO).[115] The detection limits for NO and CO were 1 nM and less than 5 nM, respectively, for tin-plated platinized platinum and platinized platinum electrodes held at a different working potentials. Meyerhoff and Cha reported that by adding an organoselenium catalyst hydrogel layer to a olytetrafluoroethylene membrane-based NO sensor, S-nitrosothiols (RSNO) could be decomposed to and quantified as NO.[116] In conjunction with endogenous RSNO, organoselenium catalyst coatings could also be used to generate NO at levels sufficient to decrease thrombosis at the surface of biomedical implants. The resulting RSNO sensor had a limit of detection of less than 0.1 μM.

Zhang et al. reported on the fabrication of a carbon fiber microelectrode modified with multiwalled carbon nanotubes (MWCNT) for measuring ascorbic acid (AA) in vivo.[117] The MWCNTs increased the electron-transfer kinetics of AA oxidation, allowing for the in vivo measurement of AA (via differential pulse voltammetry) with a wide-linear range and a detection limit of 40 μM.

Vega and co-workers modified glassy carbon electrodes with multiwalled carbon nanotubes (MWCNT) to detect tetracycline antibiotics.[118] Detection of antibiotics from 90 to 440 nM was achieved by utilizing the resulting sensor as a high pressure liquid chromatography detector. The reproducibility of such measurements was increased due to the anti-fouling nature of the MWCNTs.

Song and Swain described a method for quantifying As(III) and As(V) via anodic stripping voltammetry using Au-coated, boron-doped diamond electrodes.[119] The authors reported sub-ppb limits of detection for both arsenic species but noted that interference from Cu(II) and Pb(II) lowered the sensitivity of the method when used to analyze real samples. Compton's group was able to eliminate Cu(II) interference from As(III) measurements by utilizing the As(III) oxidation peak.[120] This method relied on the catalytic properties of a platinum nanoparticle-modified glassy carbon electrode.

Microelectrodes

The fabrication of boron-doped diamond (BDD) microelectrodes was reported by Suzuki and co-workers for the detection of dopamine.[121] The BDD was deposited onto etched tungsten wires (5 μm diameter) via microwave plasma vapor deposition. The sensors exhibited a dopamine detection limit of 50 nM and a wide-linear response range up to 100 μM, even in the presence of 1 mM ascorbic acid. The successful in vivo detection of dopamine after stimulation events was also demonstrated in a mouse model.

Several reports focused on the design of and theory governing nanopore and nanodisk electrodes. Lanyon and co-workers described the fabrication of nanopore electrode arrays using focused ion beam (FIB) milling, a technique that allows for precise control over pore size and depth.[122] Indeed, pore sizes from 150 to 400 nm and depths down to 500 nm were reproducibly milled into silicon nitride down to a platinum electrode surface. Additionally, the authors presented a thorough theoretical and experimental (pulse voltammetric) characterization of the electrode system. Zhang et al. demonstrated a simple method for fabricating nanodisk and nanopore electrodes using platinum and gold of radii down to 10 and 100 nm, respectively.[123] Electrochemically sharpened Pt and Au wires were sealed inside glass capillaries and polished to form nanodisks. A portion of the metal was then removed by etching to form nanopores. The authors placed great emphasis on the details of electrochemically sharpening the metal wire and polishing the electrode as the successful completion of these steps proved critical to reproducible fabrication. Using theory, simulation, and experimental evidence, a report from the same group described the voltammetric response of nanopore electrodes.[124] A constant diffusion-limited current may be reached only when the pore depth is 50 times greater than the diameter of the pore opening. The theory presented by the authors should prove useful to researchers seeking to design and fabricate nanopore electrodes with predictable responses. Wang and co-workers described a simple method of synthesizing polymer nanopores (20 to 120 nm radius) on the surface of a glassy carbon electrode.[125] An array of nanopores was formed in one step by spin coating a [poly(styrene)-block-poly (acrylic acid)] solution onto the electrode surface. The resulting electrode was used to successfully measure uric acid down to 0.04 μM, with minimal interference from ascorbic acid. The sensor response was linear from 0.25 to 50 μM uric acid.

Wang and Hu presented a method for fabricating nanometer-sized electrodes by electrochemically depositing poly(acrylic acid) as an insulator onto electrochemically-etched platinum wire.[126] By controlling the limiting current during the deposition step, the size of the exposed portion of the platinum was controlled and electrode surfaces as small as 3.1 nm could be fabricated.

Szamocki and co-workers reported the synthesis of macroporous ultramicroelectrodes with up to two orders of magnitude higher surface area than disk microelectrodes.[127] Electrodes were formed via electrodeposition of metal onto a template of colloidal silica particles. Following the removal of the template by dissolution in hydrofluoric acid, the resulting pore sizes range from 5 to 1000 nm. Such size may prove useful for enzymes that are unable to fit into the smaller pore sizes of mesoporous electrodes.

ELECTROCHEMICAL BIOSENSORS

The area of electrochemical biosensors continues to grow at a rapid pace based on the characteristics of fast, simple, low-cost detection capabilities inherent with biological binding. Research in this field remains focused on novel sensing strategies with specific attention to the enhancement of specificity, sensitivity, and response time. Over the last two

years, the use of nanoparticles and nanotubes has become more prominent in the fabrication of novel electrochemical biosensors.

Reviews

Pejcic et al. reviewed the role of biosensors in the detection of emerging infectious diseases (EIDs). The authors described the various types of biosensor technologies that have been used to detect EIDs, and related transduction and bioreceptor principles. Despite a host of available technologies, the development of biosensors for the detection of EIDs remains in its infancy (138 citations).[128]

Rogers detailed recent advances in biosensor techniques for environmental monitoring. The development of enzyme-, antibody-, cell-, DNA-, and receptor-based biosensors for environmental monitoring were reviewed with specific attention to the application of such sensors for measuring cytotoxicity, genotoxicity, biological oxygen demand, pathogenic bacteria, and endocrine disruption (90 citations).[129]

Wang reviewed the prospects and challenges of using electrochemical biosensors for point-of-care cancer diagnostics. The author discussed how the ability to perform cancer testing in a decentralized setting would allow for improved efficiency of cancer diagnostics and therapy monitoring, potentially facilitating early detection and enhancing patient survival rates (43 citations).[130]

Xu et al. reported on the use of advanced membrane materials including hydrogels, sol-gel-derived organic-inorganic composites, and lipid membranes to fabricate electrochemical sensors capable of measuring analytes in complex environmental and clinical samples. The authors also described recent developments in the use of biosensors for organic-phase applications and protein direct electrochemistry based on lipid membranes and nanomaterials (45 citations).[131]

Castañeda et al. reviewed electrochemical sensing of DNA using gold nanoparticles. The authors described DNA hybridization detection modes and multiple enhancement strategies for improving detection limits (87 citations). [132]

Zhao et al. examined the use of layer-by-layer (LBL) assemblies to fabricate electrochemical enzyme biosensors, immunosensors, and DNA-based sensors. The authors discussed how LBL assemblies allow for the rational design of protein-immobilized films to enhance the performance of such sensors (131 citations).[133]

Willner and Zayats reviewed the recent advances in developing electronic aptamer-based sensors (i.e., aptasensors), including electrochemical, field-effect transistor, and microgravimetric quartz crystal microbalance sensors. Methods for developing both amplified aptasensor devices and label-free aptasensors were described (57 citations).[134] Willner and coworkers also reported on the synthesis and use of biomolecule-nanoparticle hybrid systems that combine the unique electronic, photonic, and catalytic properties of nanoparticles with the specific recognition and biocatalytic properties of biomolecules. The unique functions of biomolecule-nanoparticle hybrid systems were discussed with several examples (41 citations).[135]

Asphahani and Zhang reviewed cell-based impedance biosensors as tools for the non-invasive and instantaneous detection of cell response to chemical and biological agents. Both the fabrication and measurement technologies of cell impedance sensors were highlighted, followed by a description of their application in toxin detection and anti-cancer drug screening (65 citations).[136]

Badihi-Mossberg et al. examined the recent advances in electrochemical biosensing and detection of environmental pollutants. In addition to covering the basic principles of biosensors, the authors discussed several environmental monitoring strategies (66 citations).[137]

Koncki reviewed recent developments in potentiometric biosensors for biomedical analysis. In addition to discussing the advantages, limitations, and pitfalls of bioaffinity-based potentiometric biosensors, examples of sensors for biomedical analysis were summarized (52 citations).[138]

Veetil and Ye evaluated the potential for the development of carbon nanotube-based electrochemical immunosensors. The authors discussed both the challenges to be overcome for the successful commercialization of carbon nanotube-based immunosensors and the possible impacts of such devices (97 citations).[139]

Ricci et al. reviewed new developments related to the design of immunosensors for food analysis. In addition to electrochemical biosensors, the authors described the utility of surface plasmon resonance and quartz crystal microbalance methods (139 citations).[140]

Enzyme Biosensors

Research related to enzyme-based biosensors continued at a strong pace. The following papers represent examples of the various approaches that were explored over the last two years. Beissenhirtz et al. reported the fabrication of a superoxide biosensor via engineering superoxide dismutase monomers to contain either one or two additional cysteine residues for binding to gold surfaces.[141] In this respect, the use of a promoter became unnecessary. In an amperometric biosensor approach, a superoxide dismutase-mutant electrode was successfully applied to the measurement of superoxide radicals. The sensitivity of the sensor surpassed that of previously reported cytochrome c-monolayer electrodes by ~1 order of magnitude with minimal interference from redox-active byproducts.

Stoica et al. described the fabrication of a lactose biosensor based on the efficient direct electron transfer between two newly discovered cellobiose dehydrogenases (CDH) and a solid spectrographic electrode surface.[142] The CDH was immobilized to the electrode surface via simple physisorption. Using a wall-jet amperometric cell connected on-line to a flow injection system, the sensor was able to measure lactose from 1 to 100 μM with 1 μM limit of detection at a sensitivity of 1000 μA mM^{-1} cm^{-2}, and a response time of 4 sec. The simplicity of construction and favorable analytical characteristics indicated that the CDH-based biosensor may be a good alternative to previously reported and/or commercially available lactose biosensors.

McMahon et al. reported a membrane system with the ability to regulate the dependence of oxygen for an implantable glutamate biosensor.[143] Biosensors in general must provide stable response in the presence of changing pO_2 over the range of substrate and oxygen concentrations relevant to the intended application. Biosensors for glutamate were fabricated using glutamate oxidase and poly(*o*-phenylenediamine) modified with Teflon-coated Pt wire. Multiple configurations of the polymer/enzyme layer were characterized in terms of response time, limit of detection, enzyme kinetics, sensitivity, and dependence on oxygen concentration. Enzyme loading proved key to controlling the oxygen dependence with increased glutamate oxidase loading resulting in decreased oxygen dependence.

Mizaikoff's group described a general theoretical model for competitive dual-enzyme microbiosensors based on self-assembled monolayers.[144] The model was derived from the statistical occurrence of competition between glucose oxidase and hexokinase for glucose

within the diffusion volume at the biosensor surface. While thick-film sensors depended predominately on the diffusion of the reactant through the polymer matrix, the model describing the amperometric response for monolayer enzymatic biosensors was limited by the enzymatic reaction rate. Agreement between the predicted current response and experimentally obtained sensor response was achieved at ATP concentrations between 10 and 300 μM in buffer containing physiologically relevant levels of glucose. The simplicity of this model may both reduce the development time for new dual-enzyme biosensors and be easily adapted to the calibration of a wide variety of dual-enzyme thin-film competitive biosensor schemes independent of transduction mechanism.

Leaching of the enzyme is often problematic for enzymatic biosensors and significantly reduces the lifetime of the sensor. Lin et al. reported a strategy to combat leaching by covalently linking lactate dehydrogenase to an organoalkoxysilane precursor via a carbodiimide coupling reaction prior to sol-gel processing to form the sensor membrane.[145] The resulting material was coated on a glassy carbon electrode to prepare a lactate biosensor with good long-term operational stability (~1 week).

Tian et al. reported the electrodeposition of ruthenium purple (RP) on gold microelectrodes to create sensors capable of measuring hydrogen peroxide at physiological pH ranges via electrocatalytic reduction.[146] ATP and hypoxanthine biosensors were then fabricated by depositing sol-gel films on the RP microelectrodes via chemical deposition. These RP-mediated biosensors displayed excellent selectivity over common interferences (e.g., ascorbic acid, urate, and acetaminophen), high sensitivity with a wide linear calibration range, and adequate stability. As such, RP was demonstrated to be a universal electron mediator that may be easily employed in the fabrication of highly selective oxidase-based microelectrode biosensors.

Work related to the development of implantable glucose biosensors continued at a moderate pace. The most interesting work focused on developing more robust outer sensor membranes. Moussy's group described the fabrication of a flexible glucose biosensor prepared using an epoxy-enhanced polyurethane outer membrane in order to increase the in vivo durability and lifetime.[147] Biosensors prepared using such outer membranes demonstrated excellent in situ stability for 4–8 months in buffer solutions at room temperature. The in vivo performance of such sensors in rats was also enhanced, functioning successfully up to 56 days.

Han et al. reported the fabrication of a glucose biosensor consisting of a hydrophilic polyurethane (HPU) blended with a polyvinyl alcohol/vinyl butyral copolymer (PVAB) to form an outer membrane.[148] In addition to enabling high enzyme loading, the HPU hybrid membrane displayed excellent analytical performance characteristics. Furthermore, the lifetime of such sensors was >20 days in buffer and ~3 days in human serum.

Schoenfisch's group reported the fabrication of nitric oxide-releasing glucose sensors consisting of a poly(vinylpyrrolidone) xerogel hybrid membrane.[149] The authors had previously shown the benefits of nitric oxide release when applied to in vivo sensing. Xerogel-derived nitric oxide was shown to both reduce the inflammatory response and enhance wound healing in the region surrounding the implant. A notable challenge, however, was decreased analyte diffusion through the sensor membrane upon modification with the nitric oxide donor. Doping of the hydrophilic polymer into the xerogel resulted in enhanced sensitivity to glucose without compromising the stability of the xerogel, biosensor, or polymeric nitric oxide release.

Meyerhoff's group reported the fabrication of *S*-nitrosothiol (RSNOs) biosensors via the immobilization of glutathione peroxidase (GPx), an enzyme containing selenocysteine

residues that catalytically produce nitric oxide from RSNOs.[150] *S*-nitrosothiols are among the key vehicles for storing, transferring, and delivering nitric oxide in blood and within living cells. The GPx was entrapped at the distal tip of a planar amperometric nitric oxide sensor within a microporous (poly(tetrafluoroethylene) (PTFE) gas permeable membrane. The biosensor exhibited favorable analytical performance characteristics when applied to the detection of a number of RSNO species (i.e., response times < 5 min, limit of detection ~0.2 μM, and linear response up to 6 μM). Such biosensors may prove useful for detecting relative RSNO concentrations in biological samples including whole blood.

Morales et al. reported the fabrication of a glucose oxidase-peroxidase composite biosensor for measuring bacteria speciation and concentrations.[151] Specifically, the measurement of a metabolic byproduct of bacteria (i.e., glucose) via a graphite-Teflon-glucose oxidase-peroxidase-ferrocene composite biosensor under flow injection conditions was demonstrated as useful method for simultaneously detecting and identifying bacteria. A limit of detection of 6.5 colony forming units (CFU) mL^{-1} was achieved after 3 or 7 h of incubation with no pre-concentration step. In contrast to immunological, flow cytometry, or impedimetry techniques, screening bacteria via a biosensor is more simple, rapid, and inexpensive.

Carelli et al. reported the development of an interference-free amperometric biosensor for the detection of biogenic amines in food products.[152] Commercially available diamino oxidase was used as the biocomponent and entrapped in glutaraldehyde on a electrosynthesized bilayer film. The electrode type (i.e., gold or platinum), enzyme immobilization procedure, and bilayer film each proved influential to the analytical response characteristics of the ensuing biosensor. The biosensor was then successfully applied to test for the presence of biogenic amines in several food and biological samples.

Enzyme Biosensors based on Carbon Nanotubes and Nanoparticles

Research related to biosensors employing carbon nanotubes (CNTs) and nanoparticles continued to be published at a rapid rate. A significant amount of work encompassed the use of CNTs in biosensor construction. Liu and Lin described a highly sensitive flow injection amperometric biosensor for organophosphate pesticides and nerve agents based on self-assembled acetylcholinesterase (AChE) on a CNT-modified glass carbon (GC) electrode.[153] The AChE was immobilized on the negatively charged CNT surface by alternating cationic poly(diallyldimethylammonium chloride) (PDDA) and AChE layers. This unique sandwich structure on the CNT surface provided a favorable microenvironment for the AChE, preserving enzyme bioactivity. The electrocatalytic activity of the CNT led to improved electrochemical detection of the enzymatically generated thiocholine product (e.g., low oxidation potential, higher sensitivity, and stability).

Male et al. developed an arsenite biosensor using arsenite oxidase and multi-walled CNT-modified electrodes.[154] By galvanostatically depositing the enzyme onto the CNT-modified carbon electrode, a biosensor that enabled direct electron transfer (i.e., influenced the reduction and reoxidation of the enzyme without an artificial electron-transfer mediator) was achieved. The authors were able to detect arsenite using an applied potential of 0.3 V. The response of the biosensor was linear up to 500 ppb with a 1 ppb limit of detection and minimal interference from copper.

Luo et al. demonstrated enhanced performance for a horseradish peroxidase (HRP)-based biosensor consisting of electropolymerized polyaniline (PANI) films doped with CNTs.[155] The use of CNTs enabled greater enzyme loading and enhanced enzyme stability, thereby improving the overall sensor response. The performance characteristics of the biosensor included a hydrogen peroxide detection limit of 68 nM, linear response from 0.2–19 μM, and response time < 5 sec.

Kurusu's group studied the electrochemical performance of CNT-modified oxidase-based biosensors toward hydrogen peroxide as a function of CNT type including defect-rich bamboo-structured CNTs (BCNTs), relatively defect-free hollow-structured CNTs (HCNTs), edge plane pyrolytic graphite (EPG), and traditional glassy carbon (GC).[156] The sensitivity of the BCNT paste electrode (BCNTPE) was an order of magnitude greater than both an HCNT paste electrode and a GC electrode, reflecting the larger number of defect sites for BCNT. Furthermore, the BCNTPE was able to discriminate a cathodic current for hydrogen peroxide at −0.1 V even in the presence of common interfering species.

Qu et al. reported a rapid method for loading CNT nanocomposites onto electrodes using magnets.[157] Specifically, an electrochemical sensing platform was developed via magnetic loading of CNT/nano-iron oxide (Fe_3O_4) composites on graphite-epoxy electrodes. The resulting magnetic nanocomposite combined the advantages of CNT and nano-Fe_3O_4. Chitosan was introduced into the bulk of the composite by coprecipitation to immobilize glucose oxidase, resulting in a low-potential amperometric glucose sensor.

Zhang et al. cross-linked laccase to the surface of SiO_2-coated magnetic (Fe_3O_4) nanoparticles to fabricate magnetic bio-nanoparticles.[158] The laccase-modified nanoparticles were then attached to the surface of a carbon paste electrode using a magnet to fabricate a hydroquinone biosensor. The resulting biosensor had a linear response for hydroquinone from 100 nM to 137.5 μM with a detection limit of 15 nM and ~60 sec response time.

Jena and Raj developed a NADH biosensor based on the integrated assembly of dehydrogenase enzymes and gold nanoparticles.[159] The gold nanoparticles were self-assembled on a thiol-terminated, sol–gel-derived, three-dimensional silicate network. The resulting network was shown to efficiently catalyze the oxidation of NADH and behave like a nanoelectrode ensemble. The NADH biosensors were stable, fast responding, and exhibited high sensitivity toward NADH (0.056 ± 0.001 nA nM^{-1}) with an amperometric detection limit of 5 nM.

Zhang and Hu employed poly(propyleneimine) (PPI) dendrimers to stabilize gold (Au) nanoparticles as PPI-Au nanoclusters.[160] The PPI-Au nanoclusters and myoglobin were alternatively adsorbed on the surface of pyrolytic graphite electrodes to form $\{PPI\text{-}Au/MB\}_n$ layer films. The ensuing electrodes demonstrated better electrocatalytic properties to oxygen and hydrogen peroxide reduction than $\{Au/Mb\}_n$ multilayer films containing no dendrimers and $\{PAMAM/Mb\}_n$ films assembled without Au nanoparticles.

Xu's group reported the use of thiol-functionalized poly(styrene-*co*-acrylic acid) (St-*co*-AA) nanospheres to chemisorb gold nanoparticles onto a gold electrode.[161] Horseradish peroxidase (HRP) was then immobilized on the surface of the gold nanoparticles, resulting in a sensor capable of electrocatalytically reducing hydrogen peroxide without the need for an electron mediator. The biosensor responsed linearly to hydrogen peroxide from 8.0 μM to 7.0 mM with a detection limit of 4.0 μM. Moreover, the sensor retained 97.8% of its original activity after 60 days of storage in phosphate buffer.

Shan and co-workers studied the utility of a nanoscaled calcium carbonate (nano-$CaCO_3$)-polyphenol oxidase (PPO) biocomposite for fabricating a phenol biosensor.[162] The sensor was fabricated by cross-linking the biocomposite on the surface of a glassy carbon electrode using glutaraldehyde. The three-dimensional structure, porous morphology, and hydrophilic properties of the nano-$CaCO_3$ matrix resulted in high enzyme loading and retention of enzyme activity. The analytical performance of the biosensor was characterized by a broad linear range (~10^{-9} to 10^{-5} M), short response time (<12 sec), high sensitivity (474 mA M^{-1}), subnanomolar limit of detection (0.44 nM), and long-term stability.

DNA Biosensors

Electrochemical DNA biosensors have become an attractive technique for the identification of oligonucleotides and other analytes due their ease of miniaturization, low cost, and direct readout of electronic signals. The field of DNA biosensors encompasses two distinct classes of devices: (1) sensors that detect specific sequences of DNA; and, (2) sensors that employ specific binding events of DNA to measure other analytes (e.g., proteins). Hereafter, we cover both types of DNA biosensors.

(a) Electrochemical Biosensors for Detecting DNA—Electrochemical biosensors for detecting DNA exploit the affinity of single-stranded DNA for complementary strands of DNA, and allow for both indirect and direct measurement methods. Indirect approaches are based on the detection of electroactive labels that intercalate or associate with double-stranded DNA. On the other hand, direct methods are label-free and rely mostly on the intrinsic electroactivity of nucleobases.

Ricci et al. systematically studied the factors affecting the performance of redox-modified (i.e., methylene blue or ferrocene) DNA sensors.[163] The effects of probe density and target length on the signaling properties, specificity, and response time were evaluated. The highest signal suppression was obtained when using the greatest probe densities. In addition, more suppression was achieved with longer and bulkier targets. In contrast, hybridization time (i.e., sensor response time) was reduced with increasing probe density. The specificity of the sensors was not influenced by the above factors.

A conducting polymer was employed by Malhotra and co-workers to develop ultrasensitive DNA hybridization sensors.[164] Avidine-modified polyaniline was electrochemically deposited on a Pt electrode and conjugated with single-stranded 5′-biotin end-labeled polydeoxycytidine probes and 5′-biotin end-labeled 35 base-long oligonucleotide probes to detect complementary targets. Signal was generated by exploiting both the direct electrochemical oxidation of guanine and the indirect redox electroactive indicator methylene blue. Differential pulse voltammetric hybridization detection using methylene blue allowed for a 100-fold enhanced detection limit when compared to the direct electrooxidation measurement of guanine.

Wong and Gooding introduced an electrochemical transduction system based on the redox-active intercalator, anthraquinonemonosulfonic acid (AQMS) and long-range charge transfer to produce a fast, simple, sensitive, and selective DNA biosensor.[165] The biosensor was able to detect target DNA in the sub-nanomolar range in less than 1 h. Furthermore, the in situ detection scheme enabled differentiation between complementary, non-complementary, and target DNA with single-base pair mismatches without requiring any additional steps.

Gothelf and co-workers described the electrochemical detection of specific DNA targets in the femtomolar concentration range using metal sulfide nanoparticles including CdS, ZnS, and PbS.[166] The sensor consisted of nanoparticle-DNA conjugates that were immobilized on a gold surface via hybridization with complementary DNA sequences. Upon introducing DNA targets, the DNA-nanoparticle conjugates were displaced from the surface DNA. The amount of nanoparticles remaining at the gold electrode were then measured via stripping voltammetry.

Jenkins et al. reported ferrocene-labeled hybridization probes on the gold surface of a disposable electrode.[167] The observed current densities were roughly 1000 times greater than those previously reported. The authors were able to distinguished hybridization to target oligonucleotides from competing sequences down to a single-nucleotide mismatch.

Detection limits approaching 100 fM were achieved, and suggested to be sufficient to identify genetic conditions or infections without amplification.

Great advances were made in the development of label-free methods for identifying DNA sequences. Gao et al. reported on the use of a highly ordered n-type silicon nanowire (SiNW) array for label-free detection of DNA.[168] Peptide nucleic acid (PNA) capture probe-functionalized SiNW arrays exhibited a concentration-dependent resistance change upon hybridization to complementary target DNA. The resulting DNA sensor was characterized by a wide linear dynamic range from 25 fM to 5 pM with a detection limit of 10 fM.

The Michiels group reported the development of label-free DNA biosensors sensitive to nucleotide polymorphism using impedance spectroscopy.[169] Different impedance behaviors during hybridization and denaturation were successfully observed for single-mismatch DNA and its complementary target.

Yang and co-workers reported on the use of amino-terminated fourth generation polyamidoamine (PAMAM) dendrimers as building blocks for constructing both multilayer films and linkers for immobilizing probe DNA.[170] The immobilization capacity of the probe DNA was 25 times greater for the dendrimer-modified surfaces compared to that obtained using self-assembled monolayer-based sensor surfaces (without dendrimers).

Osmium tetraoxide bipyridine [OsO_4(bipy)] has been employed previously as an electroactive reversible DNA labeler. Unfortunately, OsO_4(bipy)-modified DNA strands are unable to form double strands, limiting its utility. To overcome this problem, Flechsig and Reske employed protective strands to modify thymine-containing DNA with OsO_4(bipy) without inhibiting hybridization with probe sequences immobilized on a gold electrode.[171] The concept was successfully demonstrated by detecting short and long (25 and 120 bases, respectively) thymine-containing DNA targets with a detection limit of 3.2 nM for the long target.

Alkaline phosphatase (AP) is commonly employed for enzyme-labeled DNA hybridization assays. In the presence of nitro blue tetrazolium, AP catalyzes the substrate 3-indoxyl phosphate (3-IP) to generate indigo blue. Previous approaches for indigo blue detection are based on either its sulfonation in acidic media or its solubilization in basic media. Unfortunately, both methods require an additional step to detect the enzymatic product. Costa-Garcia and co-workers described a strategy to overcome this drawback and improve the sensitivity for DNA hybridization detection.[172] Instead of employing nitro blue tetrazolium during the AP catalytic reaction, silver ions (Ag^+) were reduced to silver (Ag^0) while generating the enzymatic product indigo blue. The deposited Ag^0 was then measured using anodic stripping voltammetry. The authors demonstrated the feasibility of this approach by detecting the determinant DNA sequence of *Streptococcus pneumoniae*, a human pathogen. The resulting genosensor showed a 14-fold improved sensitivity compared with direct voltammetric detection, enabling measurement of the target sequence down to 35 amol in 30 μL of sample volume.

The use of conducting polymers modified with DNA strands has enabled direct electrochemical detection of DNA hybridization events. The Josowicz and Kranz groups reported a label-free electrochemical detection method for short-sequence (18- and 27-mer) DNA based on a polypyrrole film deposited at a microelectrode surface.[173] The miniature DNA biosensor exhibited detection limits of 0.16 and 3.5 fmol for the 18- and 27-mer target DNAs respectively, with fast hybridization times (<10 min) for both. In addition, dissociation of the DNA duplex was achieved using acid regeneration solutions.

Plaxco and co-workers reported the fabrication of a label-free, electrochemical DNA sensor whose response was coupled to a hybridization-linked conformational change rather than merely adsorption of DNA to the sensor surface.[174] The biosensor was selective enough to detect oligonucleotides in complex, multicomponent samples such as blood serum and soil, with discrimination of single-base mismatched targets.

Li et al. reported the use of a microelectrode ($d = 10\ \mu m$) array for the detection of eight single-nucleotide mismatches by electrochemical impedance spectroscopy (EIS).[175] Randles equivalent circuits were employed to evaluate the EIS results based on differences in the electrochemical properties of duplex DNA in the presence and absence of Zn^{2+} at pH ≥ 8.6. All eight mismatches were detected by exploiting the charge-transfer resistance of B-DNA (absence of Zn^{2+} at pH ≥ 8.6) and M-DNA (presence of Zn^{2+} at pH ≥ 8.6) without relying on prior labeling of the DNA. The sensor was sufficiently sensitive to detect a single-nucleotide mismatch at a target DNA concentration of 10 fM (about 10^3 molecules per electrode) in the presence of non-target DNA (calf thymus DNA) at 10^{-8} M. In the presence of 10 $\mu g\ mL^{-1}$ bovine serum albumin, the DNA sensor also allowed for the discrimination between matched and mismatched DNA as low as 1 pM target DNA.

Hvastkovs and Buttry reported the synthesis of a new electroactive bis-intercalator, $NI(CH_2)_3V^{2+}(CH_2)_6V^{2+}(CH_2)_3NI$ (where NI = naphthylimide and V^{2+} = 4,4′-bipyridinium; NIV), and its utility for DNA hybridization detection.[176] The NIV bio-intercalator dissociated significantly (i.e., 160 times) more slowly from dsDNA than from ssDNA, allowing effective disctimination between dsDNA and ssDNA. The electronic signal was generated by reduction of V^{2+} to V^+ during cyclic voltammetry and chronocoulometry.

Miranda-Castro et al. described an electrochemical genosensor for detecting *Legionella pneumophila* using an immobilized hairpin probe via a sandwich-hybridization assay combined with enzymatic amplification.[177] The sensor showed high sensitivity towards a 52-mer DNA sequence characteristic for *L. pneumophila* with a detection limit of 340 pM and no cross-reactivity for *L. longbeachae*.

Tsang and co-workers described a label-free DNA sensor by employing highly conductive praseodymium oxide (Pr_6O_{11}) nanoparticles modified with single stranded (ss)-DNA sequences.[178] The capacitance of the Pr_6O_{11} represented in an equivalent circuit was shown to be influenced by changes in local environment (i.e., chemical concentration and immobilization events). The authors went on to demonstrate the operation of a label-free, highly sensitive DNA sensor via impedance changes observed upon hybridization with complimentary target DNA.

(b) DNA-Based Biosensors for Other Analytes—The use of artificial nucleic acid ligands (i.e., aptamers) for biosensing has garnered great attention due to simple isolation and modification, tailored binding affinity, and resistance against denaturation. Tuñón-Blanco and co-workers reported the use of fully 2′-O-methylated RNA sequences as recognition elements for a competitive impedimetric assay of aminoglycoside neomycin B in whole milk.[179] The authors showed that the modified endonuclease-resistant RNA aptamer allowed for the fabrication of a reusable aptasensor with excellent selectivity over paromomycin, which differs from neomycin B by one functional group.

Cheng et al. described a label-free, electrochemical lysozyme aptasensor based on anti-lysozyme DNA sequences immobilized on a gold surface.[180] Lysozyme binding with the aptamer was detected by cyclic voltammetry. Increasing the lysozyme concentrations in the sample solution decreased the voltammetric signal of surface-bound $[Ru(NH_3)_6]^{3+}$ as a result of its increased electrostatic interaction with the DNA-modified surface. The aptamer-

based biosensor was capable of detecting physiological concentrations of lysozyme from 0.5 to 50 μg mL^{-1}.

The Wang group reported on an electrochemical aptamer biosensor capable of detecting multiple analytes simultaneously.[181] The sensor was prepared by modifying a gold surface with a mixed monolayer of thiolated aptamers coupled with protein-quantum dot conjugates. Stripping voltammetry was used to distinguish the composition of encoding quantum dots (i.e., cadmium sulfide, zinc sulfide, copper sulfide, and lead sulfide) upon their selective binding to protein targets. The displacement of quantum dot proteins by sample solution proteins allowed for detection limits approaching the subpicomolar (attomole) level.

To measure cocaine concentrations directly from blood, Baker et al. reported the development of an electronic aptamer-based sensing platform utilizing electrochemical interrogation of a structure-switching aptamer to generate signals based on binding-induced folding.[182] In addition to being reusable, the sensors were characterized as having a high sensitivity for cocaine with a detection limit of 10 μM and a response time ($t_{97\%}$) of <80 sec.

Xiang et al. described an ultrasensitive aptamer-based thrombin biosensor via polymerase chain reaction (PCR) signal amplification.[183] The detection scheme involved the following steps: 1) binding of a biotinylated primary thrombin aptamer (TBA-1) to the streptavidin-coated magnetic beads; 2) capture of the thrombin analyte; 3) binding of a guanine-rich secondary thrombin aptamer (TBA-2) to the captured thrombin; 4) release of TBA-2 under acidic conditions; 5) PCR amplification of TBA-2; 6) purification and acidic depurination of the amplified product; and, 7) adsorptive stripping measurements of the free guanine bases at a graphite electrode. Although the sensing protocol required two aptamers and multiple processes to obtain a signal, the detection limit for thrombin was 0.2 pg mL^{-1} (5.4 fM). Using a similar sandwich assay to amplify the electronic signal, Zhou et al. introduced the concept of aptamer-based rolling circle amplification (aptamer-RCA).[184] The aptamer-primer sequence was used to bridge the communication gap between DNA and proteins in RCA reactions where hundreds of copies of a circular DNA template may be generated. Protein targets in the sample solution were first bound to the specific antibody immobilized on a gold surface. The aptamer-primer complex was then hybridized with captured proteins, and then ligated with the padlock probe. In the final step, the amplified sequences were detected via an enzymatic silver deposition assay during anodic stripping voltammetry. The sensor exhibited extremely high sensitivity in the detection of platelet-derived growth factor B-chain with a detection limit of 10 fM.

Zheng et al. described for the fabrication of an ultrasensitive electrochemical aptasensor for the detection of thrombin via the formation of thiocyanuric acid (TCA)/gold nanoparticles.[185] Signal amplification consisted of a sandwich structure of magnetic nanoparticle-immobilized aptamerI/thrombin/gold nanoparticle-labeled aptamerII. The TCA capping agent was then used to create a dense, network-like structure. The thrombin sensor exhibited a detection limit of 7.82 aM at S/N = 3, even in the presence of other proteins such as bovine serum albumin (BSA). The sensitivity of the electrochemical aptasensor was attributed to the aggregation of such network-like TCA/gold nanoparticles.

Zhang's group reported on an electrochemical DNA biosensor for the detection of hepatitis B virus.[186] A label-free 21-mer ssDNA sequence complementary to a portion of the hepatitis B virus was immobilized on a glassy carbon electrode. Differential pulse voltammetry where [Cu(2,9-dimethyl-1,10-phenanthroline)(H_2O)Cl_2] was used as the electroactive DNA hybridization indicator resulted in the measurement of hepatitis B virus from 88.2 to 882 nM with a detection limit of 70 nM.

Studying the damage to DNA by oxidative stress and the protective effects of antioxidants to circumvent such damage is of great interest to gene-related research. Girault and co-workers described a new approach to monitor redox antioxidant properties using an electrochemical DNA sensor where double-stranded (ds)-DNA was immobilized on screen-printed carbon/TiO_2 electrodes.[187] Tris-2,2′-bipyridine ruthenium(II) [$Ru(bpy)_3^{3+}$], an electrogenerated mediator, was used as a ds-DNA redox oxidant. DNA damage was then monitored by detecting the oxidation currents of $Ru(bpy)_3^{2+}$ via square wave voltammetry.

Immunosensors

Electrochemical immunosensors remained a popular area of study due to their inherently high sensitivity. The majority of papers concerning these sensors involved new substrates and better immobilization methods to improve sensitivity and linear range. For example, Dong et al. reported the use of electropolymerized pyrrolepropylic acid (PPA) to create a sandwich-type alkaline phosphatase-catalyzing electrochemical immunosensor sensitive to IgG.[188] In addition to high porosity and hydrophilicity, the PPA had a high density of carboxyl groups that could be used to covalently attach proteins, leading to improved detection sensitivity compared to conventional enzyme entrapment methods. Indeed, the detection limit and dynamic range for IgG in PBS buffer (pH 7.4) was 100 pg mL^{-1} and 5 orders of magnitude, respectively, for immunosensors fabricated using covalently immobilized anti-mouse IgG in PPA and *p*-aminophenyl phosphate as the substrate. In carbonate-bicarbonate buffer (pH 9.6), the detection limit was further improved to 20 pg mL^{-1}.

Ionescu and co-workers developed an amperometric immunosensor for the detection of West Nile virus (WNV) antibodies.[189] Specifically, T7 phages to WNV were modified with an additional peptide sequence taken from the virus and used as an antigen. A phage-amphiphilic pyrrole ammonium mixture was then adsorbed on an electrode surface and electropolymerized to entrap the phages in a polypyrrole film. Upon incubation with a secondary peroxidase-labeled antibody, the immunosensors were capable of measuring WNV-antibodies amperometrically via the reduction of the enzymically generated quinine in the presence of hydroquinone and hydrogen peroxide. WNV-antibody dilutions down to a titer of $1:10^7$ were detected using an optimized immunosensor configuration. The immunosensor was also capable of efficient regeneration by glycine treatment.

Wilson and Nie described a novel amperometric biosensor for performing simultaneous electrochemical multianalyte immunoassays.[190] The authors patterned eight iridium oxide sensing electrodes (0.78 mm^2 each), an iridium counter electrode, and a Ag/AgCl reference electrode on a glass substrate. Four different capture antibodies were immobilized on the sensing electrodes via adsorption. The simultaneous detection of goat IgG, mouse IgG, human IgG, and chicken IgG was demonstrated with detection limits of 3 ng mL^{-1} for each protein, and negligible amperometric cross-talk between electrodes due to their spatial separation. The precision of such sensors was excellent (1.9–8.2% interassay coefficients of variation). The performance of the biosensor array was comparable in performance to commercial single-analyte ELISAs.

Zacco et al. developed an electrochemical immunosensing strategy for the detection of atrazine using magnetic beads modified with anti-atrazine antibodies.[191] The immunoassay for atrazine was a direct competitive assay using a peroxidase tracer as the enzymatic label. Upon completion of the immunochemical reactions, the magnetic beads were captured by a magnetosensor made of graphite-epoxy composite that also served as the electrochemical transducer. The detection limit for atrazine from orange juice samples was 0.027 nmol L^{-1}.

To lower detection limits, Das and co-workers focused on signal amplification and noise reduction.[192] The authors designed an electrochemical immunosensor in which signal amplification was achieved using *p*-aminophenol redox cycling by hydrazine. The *p*-aminophenol produced in a sandwich-type heterogeneous immunosensor for mouse IgG whereby alkaline phosphatase converts *p*-aminophyenyl phosphate to *p*-aminophenol was electrooxidized at an indium tin oxide (ITO) electrode modified with a partially ferrocenyltethered dendrimer. The oxidized product, *p*-quinone imine, was reduced back to *p*-aminophenol by hydrazine and the *p*-aminophenol electroxidized again to, *p*-quinone imine. Low background current (i.e., noise) was achieved by coupling only a small amount of ferrocene to the dendrimer. The detection limit and linear range of the immunosensor for mouse IgG was 100 fg mL^{-1} and 9 orders of magnitude, respectively.

Lu et al. described the development of an electrochemical, recombinant Fab fragment-based immunosensor for the detection of testosterone in bovine urine.[193] A testosterone conjugate was immobilized on the surface of screen-printed electrodes. Anti-testosterone Fab fragment recognition followed, with the use of an IgG-horseradish peroxidase conjugate allowing for the determination of the degree of competition. The product of 3,3′, 5,5′-tetramethylbenzidine catalysis was measured via chronoamperometry at a potential of +100 mV. The Fab-based testosterone immunosensor exhibited a linear range of 300–40,000 pg mL^{-1} with a detection limit of 90 ± 13 pg mL^{-1}. Furthermore, the sensor allowed for testosterone determination in bovine urine directly after dilution without extraction and hydrolysis.

Recent work was also focused on the use of nanoparticles and nanotubes to improve immunosensor sensitivity. Examples ranged from antibody-coated carbon nanotubes to nanoparticle–promoted catalysis. For instance, Viswanathan and co-workers reported an electrochemical immunosensor for cholera toxin using liposomes and poly(3,4-ethylenedioxythiophene)-coated carbon nanotubes.[194] The liposomes, encapsulated with potassium ferrocyanide and functionalized with ganglioside, served as recognition labels for cholera toxin. The sensing interface consisted of monoclonal antibody (against the B subunit of cholera toxin) linked to poly(3,4-ethylenedioxythiophene)-coated Nafion-supported multi-walled carbon nanotube films on glassy carbon electrodes. A sandwich-type assay was employed on the electronic transducer consisting of toxin bound to the anti-cholera toxin antibody and the ganglioside-functionalized liposome. Potassium ferrocyanide released from the electrode-bound liposomes via lyses in a methanolic solution of Triton X-100 was measured by adsorptive square-wave stripping voltammetry. The response of the sensor was linear over 7 orders of magnitude with a detection limit of 10^{-16} g cholera toxin.

Tang et al. reported the development of an electrochemical-based immunosensor for the determination of carcinoma antigen 125 (CA125) using CA125 antibody-modified gold nanoparticles immobilized on a thionine-modified carbon paste interface.[195] Prior to the immunoassay, the carbon paste electrodes were treated to create surfaces rich in carboxylic acid groups to enable covalent linkage with the thionine molecules (via amines). A direct immunoassay format was used to measure CA125 via changes in the electrochemical current before and after antigen-antibody reaction. The immunosensor was sensitive from 10 to 30 U mL^{-1} with a detection limit of 1.8 U mL^{-1}.

Chumbimuni-Torres et al. reported the use of potentiometry for ultrasensitive nanoparticle-based detection of protein binding.[196] A gold nanoparticle-labeled antibody was employed as the secondary antibody in a sandwich immunoassay format. Upon the formation of the sandwich complex, silver was catalytically deposited on the gold, then dissolved and measured using a silver ion-selective microelectrode. This clever approach resulted in a detection limit of approximately 12.5 pmol of mouse IgG antigen from a 50 μL sample. In a

similar approach, Chen and co-workers reported the use of silver nanoparticles to amplify an electrochemical sandwich immunoassay.[197] Silver nanoparticles were biocatalytically deposited and then enlarged using a silver-enhancer solution following the formation of the antibody-antigen complex. The silver was then dissolved and quantified by anodic stripping voltammetry. The anodic stripping peak current measured was proportional to the antibody (human IgG) concentration. The detection limit and dynamic range of this approach was 0.03 ng mL^{-1} and 0.1–10 ng mL^{-1}, respectively.

Wang et al. demonstrated the use of electroactive poly(guanine)-functionalized silica nanoparticles as labels to detect a tumor necrosis factor-alpha (TNF-α) biomarker via a sandwich immunoassay.[198] First, the poly[G]-functionalized silica nanoparticles were immobilized on an electrode to create a guanine-enriched surface. A mediator, $Ru(bpy)_3^{3+}$, was used to catalytically oxidize guanine. Redox currents were measured by square wave voltammetry. The combination of poly[G]-functionalized silica nanoparticles and catalytic oxidation of guanine led to excellent sensitivity with a detection limit of 5.0×10^{-11} g mL^{-1} (2.0 pM) for TNF-α.

Ou et al. reported the use of a layer-by-layer assembly of gold nanoparticles, thionine-modified multi-walled carbon nanotubes, and chitosan on a 3-mercaptopropanesulfonic, sodium salt-modified gold electrode surface to create a highly sensitive and label-free carcinoembryonic antigen (CEA) immunosensor.[199] The stepwise layer-by-layer assembly process of the electroactive species on the electrode surface was characterized by cyclic voltammetry and transmission electron microscopy. The immunosensor was highly sensitive to CEA with a detection limit of 0.01 ng mL^{-1} (S/N of 3) and linear range with two concentration ranges of 0.5 to 15.0 ng mL^{-1} and 15.0 to 200.0 ng mL^{-1}, respectively.

Rusling and co-workers reported on a novel amplification strategy based on the use of single-wall carbon nanotubes (SWNTs) for developing highly sensitive and selective electrochemical immunosensors.[200] Increased sensitivity was achieved by using multi-labeled bioconjugates consisting of horseradish peroxidase (HRP) labels and secondary antibodies (Ab_2) linked to multiple-wall carbon nanotubes (MWNT). As a model target, prostate specific antigen (PSA), a cancer biomarker, was detected in serum and tissue lysates. The immunosensor provided a detection limit of 4 pg mL^{-1} (100 aM) in 10 μL of undiluted calf serum.

CONCLUSIONS

The development of chemical sensors remains one of the most active areas of analytical chemistry research as evidenced by the thousands of sensor manuscripts published during the past two years. Moreover, electrochemical sensors represent the most rapidly growing class of chemical sensors due to their miniaturization potential, design simplicity, low cost, and direct readout of transduction signals. The field of potentiometric sensors has undergone a steady transformation in the past two years. Since the discovery that the detection limit of ion-selective membrane electrodes may be lowered down to picomolar levels by tuning the inner filling solution, much effort has been devoted to expanding the concept to develop miniaturized all-solid-state membrane electrodes. Indeed, using a solid contact layer between the metal electrode and the sensing membrane eliminates the influence of the inner filling solution on sensor performance. Solid contact consisting of conducting polymers has also been employed to achieve highly stable sensor response. Such response is attributed to the improved interface between the membrane and the electrode. One new trend in potentiometric sensor research is the use of pulstrodes combining potentiometric and voltammetric methods. For example, pulse chronopotentiometry has been developed to improve inherent limitations of classical potentiometry such as low sensitivity and the need

for a precise, reliable reference electrode with a liquid junction. Although significant advances have been made in fabricating miniaturized reference electrodes, their utility is still limited by poor lifetime and insufficient stability in high ionic strength physiological samples.

Research in the field of voltammetric sensors has continued at a rapid pace. The miniaturization of sensor platforms allowed for the expansion of their applications into a number of areas. Improvements in the biocompatibility of voltammetric sensing systems have expanded their potential to long-term, in vivo monitoring. The use of more biocompatible polymers and surface modifications with hydrophilic copolymers, anticoagulants (e.g., heparin), and membranes that release bioactive substances (e.g., nitric oxide) have been widely explored. The use of voltammetric sensors for environmental monitoring applications has increased due to the portability, low cost, and ability of such devices to detect contaminants at trace levels. Additionally, the incorporation of nanomaterials (e.g., nanoparticles and carbon nanotubes) into sensor designs has resulted in sensors with improved analytical performance. Another major trend related to voltammetric sensor research has involved the replacement of toxic mercury-based electrodes with environmentally benign materials such as bismuth films.

The design and fabrication of electrochemical biosensors continues to prove useful for medical, pharmacological, industrial, and environmental applications. Glucose-biosensor research remains active with a continued focus on both in vitro and in vivo glucose measurements in biological samples. The development of enzyme-based sensors for detecting other biologically-relevant analytes such as nitrosothiols and superoxide has been initiated and represents an exciting new area of biosensor research for studying disease. New concepts and sensor platforms related to sensor performance, biocompatibility, and enzyme stability have also been explored. In the area of affinity biosensors based on DNA hybridization or immunological recognition events, a number of electrochemical detection schemes for measuring important analytes have been described. Both label (e.g., enzymes and electroactive intercalators) and label-free approaches have been employed to improve sensor design. The synthesis of nanostructured labels including quantum dots, metal clusters, magnetic particles, and carbon nanotubes to improve sensor performance represents a burgeoning area of research.

Indeed, significant advances have been made in several areas related to the design and application of electrochemical sensors. We believe future work will continue to focus on further improving sensor response, selectivity, lifetime, and miniaturization while maintaining design simplicity and reduced fabrication costs.

LITERATURE CITED

1. Bakker E, Pretsch E. Angew Chem, Int Ed. 2007; 46:5660–5668.
2. Pretsch E. TrAC, Trends Anal Chem. 2007; 26:46–51.
3. De Marco R, Clarke G, Pejcic B. Electroanalysis. 2007; 19:1987–2001.
4. Bobacka J. Electroanalysis. 2006; 18:7–18.
5. Michalska A. Anal Bioanal Chem. 2006; 384:391–406. [PubMed: 16365779]
6. Gavalas VG, Berrocal MJ, Bachas LG. Anal Bioanal Chem. 2006; 384:65–72. [PubMed: 16132141]
7. Si P, Chi Q, Li Z, Ulstrup J, Moller PJ, Mortensen J. J Am Chem Soc. 2007; 129:3888–3896. [PubMed: 17355132]
8. Ge C, Armstrong NR, Saavedra SS. Anal Chem. 2007; 79:1401–1410. [PubMed: 17297939]
9. Imokawa T, Williams K-J, Denuault G. Anal Chem. 2006; 78:265–271. [PubMed: 16383336]
10. Kim H-S, Kim D-H, Kim KS, Choi J-H, Choi H-J, Kim S-H, Shim JH, Cha GS, Nam H. Talanta. 2007; 71:1986–1992. [PubMed: 19071552]

11. Gupta VK, Singh AK, Khayat MA, Gupta B. Anal Chim Acta. 2007; 590:81–90. [PubMed: 17416226]
12. Marques de Oliveira IA, Pla-Roca M, Escriche L, Casabo J, Zine N, Bausells J, Teixidor F, Crespo E, Errachid A, Samitier J. Electrochim Acta. 2006; 51:5070–5074.
13. Ardakani MM, Jalayer M, Naeimi H, Heidarnezhad A, Zare HR. Biosens Bioelectron. 2006; 21:1156–1162. [PubMed: 15925504]
14. Seguí M-J, Lizondo-Sabater J, Martínez-Máñez R, Sancenón F, Soto J. Talanta. 2006; 68:1182–1189. [PubMed: 18970449]
15. Mitchell-Koch JT, Pietrzak M, Malinowska E, Meyerhoff ME. Electroanalysis. 2006; 18:551–557.
16. Pietrzak M, Meyerhoff ME, Malinowska E. Anal Chim Acta. 2007; 596:201–209. [PubMed: 17631098]
17. Kharitonov SV. Russ Chem Rev. 2007; 76:361–395.
18. Santini AO, Pezza HR, Pezza L. Talanta. 2006; 68:636–642. [PubMed: 18970368]
19. D'Agostino G, Alberti G, Biesuz R, Pesavento M. Biosens Bioelectron. 2006; 22:145–152. [PubMed: 16815698]
20. Yin X, Ding J, Zhang S, Kong J. Biosens Bioelectron. 2006; 21:2184–2187. [PubMed: 16303298]
21. Javanbakht M, Badiei A, Ganjali MR, Norouzi P, Hasheminasab A, Abdouss M. Anal Chim Acta. 2007; 601:172–182. [PubMed: 17920389]
22. Shim JH, Kim J, Cha GS, Nam H, White RJ, White HS, Brown RB. Anal Chem. 2007; 79:3568–3574. [PubMed: 17411008]
23. Szigeti Z, Malon A, Vigassy T, Csokai V, Grün A, Wygladacz K, Ye N, Xu C, Chebny VJ, Bitter I, Rathore R, Bakker E, Pretsch E. Anal Chim Acta. 2006; 572:1–10. [PubMed: 17723454]
24. Radu A, Peper S, Bakker E, Diamond D. Electroanalysis. 2007; 19:144–154.
25. Malon A, Vigassy T, Bakker E, Pretsch E. J Am Chem Soc. 2006; 128:8154–8155. [PubMed: 16787077]
26. Rubinova N, Chumbimuni-Torres K, Bakker E. Sens Actuators, B. 2007; 121:135–141.
27. Sutter J, Pretsch E. Electroanalysis. 2006; 18:19–25.
28. Szigeti Z, Vigassy T, Bakker E, Pretsch E. Electroanalysis. 2006; 18:1254–1265. [PubMed: 20336172]
29. Wanichacheva N, Soto ER, Lambert CR, McGimpsey WG. Anal Chem. 2006; 78:7132–7137. [PubMed: 17037912]
30. Malon A, Bakker E, Pretsch E. Anal Chem. 2007; 79:632–638. [PubMed: 17222030]
31. Ngeontae W, Xu Y, Xu C, Aeungmaitrepirom W, Tuntulani T, Pretsch E, Bakker E. Anal Chem. 2007; 79:8705–8711. [PubMed: 17929899]
32. Lingenfelter P, Bedlechowicz-Silwakowska I, Sokalski T, Maj-Zurawska M, Lewenstam A. Anal Chem. 2006; 78:6783–6791. [PubMed: 17007497]
33. Michalska A, Wojciechowski M, Wagner B, Bulska E, Maksymiuk K. Anal Chem. 2006; 78:5584–5589. [PubMed: 16878899]
34. Calvo D, Bartroli J, del Valle M. Electrochim Acta. 2006; 51:1569–1575.
35. Elsen HA, Slowinska K, Hull E, Majda M. Anal Chem. 2006; 78:6356–6363. [PubMed: 16970309]
36. Chumbimuni-Torres KY, Rubinova N, Radu A, Kubota LT, Bakker E. Anal Chem. 2006; 78:1318–1322. [PubMed: 16478128]
37. Lindfors T, Aarnio H, Ivaska A. Anal Chem. 2007; 79:8571–8577. [PubMed: 17929876]
38. Lai C-Z, Fierke MA, Stein A, Bühlmann P. Anal Chem. 2007; 79:4621–4626. [PubMed: 17508716]
39. Gabrielli C, Hemery P, Liatsi P, Masure M, Perrot H. Electrochim Acta. 2006; 51:1704–1712.
40. Makarychev-Mikhailov S, Shvarev A, Bakker E. Anal Chem. 2006; 78:2744–2751. [PubMed: 16615788]
41. Gemene KL, Shvarev A, Bakker E. Anal Chim Acta. 2007; 583:190–196. [PubMed: 17386545]
42. Perera H, Shvarev A. J Am Chem Soc. 2007; 129:15754–15755. [PubMed: 18052379]

43. Xu Y, Xu C, Shvarev A, Becker T, Marco RD, Bakker E. Anal Chem. 2007; 79:7154–7160. [PubMed: 17711298]
44. Perera H, Fordyce K, Shvarev A. Anal Chem. 2007; 79:4564–4573. [PubMed: 17488093]
45. Liao W-Y, Chou T-C. Anal Chem. 2006; 78:4219–4223. [PubMed: 16771553]
46. Kwon N-K, Lee K-S, Won M-S, Shim Y-B. Analyst. 2007; 132:906–912. [PubMed: 17710266]
47. Kakiuchi T, Yoshimatsu T, Nishi N. Anal Chem. 2007; 79:7187–7191. [PubMed: 17708675]
48. Hashimoto M, Upadhyay S, Kojima S, Suzuki H, Hayashi K, Sunagawa K. J Electrochem Soc. 2006; 153:H155–H160.
49. Kim SK, Lim H, Chung TD, Kim HC. Sens Actuators, B. 2006; 115:212–219.
50. Han J-H, Park S, Boo H, Kim HC, Nho J, Chung TD. Electroanalysis. 2007; 19:786–792.
51. Ghilane J, Hapiot P, Bard AJ. Anal Chem. 2006; 78:6868–6872. [PubMed: 17007508]
52. Nagy G, Nagy L. Anal Lett. 2007; 40:3–38.
53. Ordeig O, del Campo J, Muñoz FX, Banks CE, Compton RG. Electroanalysis. 2007; 19:1973–1986.
54. Riu J, Maroto A, Rius FX. Talanta. 2006; 69:288–301. [PubMed: 18970568]
55. Luo X, Morrin A, Killard AJ, Smyth MR. Electroanalysis. 2006; 18:319–326.
56. Frost M, Meyerhoff ME. Anal Chem. 2006; 78:7370–7377. [PubMed: 17128516]
57. Shin JH, Schoenfisch MH. Analyst. 2006; 131:609–615. [PubMed: 16795923]
58. Wang J. Electroanalysis. 2007; 19:415–423.
59. Renedo OD, Alonso-Lomillo MA, Martinez MJA. Talanta. 2007; 73:202–219. [PubMed: 19073018]
60. Barek J, Fischer J, Navrátil T, Pecková K, Yosypchuk B, Zima J. Electroanalysis. 2007; 19:2003–2014.
61. Esteban M, Ariño C, Díaz-Cruz JM. TrAC, Trends Anal Chem. 2006; 25:86–92.
62. Chang B-Y, Park S-M. Anal Chem. 2006; 78:1052–1060. [PubMed: 16478095]
63. Dudko OK, Szabo A, Ketter J, Wightman RM. J Electroanal Chem. 2006; 586:18–22.
64. Anastassiou CA, Ducros N, Parker KH, O'Hare D. Anal Chem. 2006; 78:4383–4389. [PubMed: 16808445]
65. Chang B-Y, Park S-M. Anal Chem. 2007; 79:4892–4899. [PubMed: 17530816]
66. Sun P, Mirkin MV. Anal Chem. 2006; 78:6526–6534. [PubMed: 16970330]
67. Morf WE, Koudelka-Hep M, Rooij NFd. J Electroanal Chem. 2006; 590:47–56.
68. Bieniasz LK, Rabitz H. Anal Chem. 2006; 78:1807–1816. [PubMed: 16536415]
69. Jones SEW, Chevallier FG, Paddon CA, Compton RG. Anal Chem. 2007; 79:4110–4119. [PubMed: 17469796]
70. Hocevar SB, Svancara I, Ogorevc B, Vytras K. Anal Chem. 2007; 79:8639–8643. [PubMed: 17949057]
71. Kefala G, Economou A. Anal Chim Acta. 2006; 576:283–289. [PubMed: 17723644]
72. Hutton EA, Hočevar SB, Mauko L, Ogorevc B. Anal Chim Acta. 2006; 580:244–250. [PubMed: 17723780]
73. Prior C, Lenehan CE, Walker GS. Anal Chim Acta. 2007; 598:65–73. [PubMed: 17693308]
74. Berduque A, Lanyon YH, Beni V, Herzog G, Watson YE, Rodgers K, Stam F, Alderman J, Arrigan DWM. Talanta. 2007; 71:1022–1030. [PubMed: 19071408]
75. van den Berg CMG. Anal Chem. 2006; 78:156–163. [PubMed: 16383323]
76. Salaün P, van den Berg CMG. Anal Chem. 2006; 78:5052–5060. [PubMed: 16841929]
77. Xiao L, Dietze W, Nyasulu F, Mibeck BAF. Anal Chem. 2006; 78:5172–5178. [PubMed: 16841944]
78. Baś B. Anal Chim Acta. 2006; 570:195–201. [PubMed: 17723399]
79. Yong L, Armstrong KC, Dansby-Sparks RN, Carrington NA, Chambers JQ, Xue Z-L. Anal Chem. 2006; 78:7582–7587. [PubMed: 17073430]
80. Anderson EC, Fritsch I. Anal Chem. 2006; 78:3745–3751. [PubMed: 16737232]

81. Wang J, Thongngamdee S, Lu D. Anal Chim Acta. 2006; 564:248–252.
82. Chun K, Kim TH, Lee O-S, Hirose K, Chung TD, Chung DS, Kim H. Anal Chem. 2006; 78:7597–7600. [PubMed: 17073433]
83. El-Hady DA, Seliema MM, Gotti R, El-Maali NA. Sens Actuators, B. 2006; 113:978–988.
84. Ivandini TA, Sato R, Makide Y, Fujishima A, Einaga Y. Anal Chem. 2006; 78:6291–6298. [PubMed: 16970300]
85. Donner S, Li H-W, Yeung ES, Porter MD. Anal Chem. 2006; 78:2816–2822. [PubMed: 16615798]
86. Chicharro M, Bermejo E, Moreno M, Sánchez A, Zapardiel A, Rubio-Marcos F, Villegas M. Electroanalysis. 2006; 18:1614–1619.
87. Maleki N, Safavi A, Tajabadi F. Anal Chem. 2006; 78:3820–3826. [PubMed: 16737243]
88. Khan MAK, Long Y-T, Schatte G, Kraatz H-B. Anal Chem. 2007; 79:2877–2884. [PubMed: 17319647]
89. Salimi A, Roushani M, Haghighi B, Soltanian S. Biosens Bioelectron. 2006; 22:220–226. [PubMed: 16931295]
90. Yang C, Lu Q, Hua S. Electroanalysis. 2006; 18:2188–2193.
91. Wu Y, Rojas AP, Grifth GW, Skrzypchak AM, Lafayette N, Bartlett RH, Meyerhoff ME. Sens Actuators, B. 2007; 121:36–46.
92. Ulyanova YV, Blackwell AE, Minteer SD. Analyst. 2006; 131:257–261. [PubMed: 16440091]
93. Huang H-C, Huang S-Y, Lin C-I, Lee Y-D. Anal Chim Acta. 2007; 582:137–146. [PubMed: 17386485]
94. Carrington NA, Yong L, Xue Z-L. Anal Chim Acta. 2006; 572:17–24. [PubMed: 17723456]
95. Su L, Gao F, Mao L. Anal Chem. 2006; 78:2651–2657. [PubMed: 16615776]
96. Yogeswaran U, Thiagarajan S, Chen S-M. Anal Biochem. 2007; 365:122–131. [PubMed: 17428433]
97. Dai X, Wildgoose GG, Compton RG. Analyst. 2006; 131:1241–1247. [PubMed: 17066193]
98. Zhang T, Nix MB, Yoo B-Y, Deshusses MA, Myung NV. Electroanalysis. 2006; 12:1153–1158.
99. Wang J, Tangkuaram T, Loyprasert S, Vazquez-Alvarez T, Veerasai W, Kanatharana P, Thavarungkul P. Anal Chim Acta. 2007; 581:1–6. [PubMed: 17386417]
100. Li J, Lin X-Q. Anal Chim Acta. 2007; 596:222–230. [PubMed: 17631100]
101. Hrapovic S, Liu Y, Luong JHT. Anal Chem. 2007; 79:500–507. [PubMed: 17222013]
102. Sun N, Guan L, Shi Z, Li N, Gu Z, Zhu Z, Li M, Shao Y. Anal Chem. 2006; 78:6050–6057. [PubMed: 16944883]
103. Forzani ES, Li X, Tao N. Anal Chem. 2007; 79:5217–5224. [PubMed: 17563117]
104. Hrapovic S, Majid E, Liu Y, Male K, Luong JHT. Anal Chem. 2006; 2006
105. Zhang H-X, Cao A-M, Hu J-S, Wan L-J, Lee S-T. Anal Chem. 2006; 78:1967–1971. [PubMed: 16536434]
106. Chen J-C, Shih J-L, Liu C-H, Kuo M-Y, Zen J-M. Anal Chem. 2006; 78:3752–3757. [PubMed: 16737233]
107. Pacsial-Ong EJ, McCarley RL, Wang W, Strongin RM. Anal Chem. 2006; 78:7577–7581. [PubMed: 17073429]
108. Chen J, He Z, Liu H, Cha C. J Electroanal Chem. 2006; 588:324–330.
109. Zhou M, Ding J, Guo L-p, Shang Q-k. Anal Chem. 2007; 79:5328–5335. [PubMed: 17555298]
110. Lyon JL, Stevenson KJ. Anal Chem. 2006; 78:8518–8525. [PubMed: 17165848]
111. Miah MR, Ohsaka T. Anal Chem. 2006; 78:1200–1205. [PubMed: 16478112]
112. Oh BK, Robbins ME, Schoenfisch MH. Analyst. 2006; 131:48–54. [PubMed: 16365662]
113. Griveau S, Dumézy C, Séguin J, Chabot GG, Scherman D, Bedioui F. Anal Chem. 2007; 79:1030–1033. [PubMed: 17263331]
114. Krylov AV, Lisdat F. Electroanalysis. 2007; 19:23–29.
115. Lee Y, Kim J. Anal Chem. 2007; 79:7669–7675. [PubMed: 17877421]
116. Cha W, Meyerhoff ME. Langmuir. 2006; 22:10830–10836. [PubMed: 17129067]

117. Zhang M, Liu K, Xiang L, Lin Y, Su L, Mao L. Anal Chem. 2007; 79:6559–6565. [PubMed: 17676820]
118. Vega D, Agüí L, González-Cortés A, Yáñez-Sedeño P, Pingarrón JM. Anal Bioanal Chem. 2007; 389:951–958. [PubMed: 17671781]
119. Song Y, Swain GM. Anal Chem. 2007; 79:2412–2420. [PubMed: 17302386]
120. Dai X, Compton RG. Analyst. 2006; 131:516–521. [PubMed: 16568168]
121. Suzuki A, Ivandini TA, Yoshimi K, Fujishima A, Oyama G, Nakazato T, Hattori N, Kitazawa S, Einaga Y. Anal Chem. 2007; 79:8608–8615. [PubMed: 17918970]
122. Lanyon YH, Marzi GD, Watson YE, Quinn AJ, Gleeson JP, Redmond G, Arrigan DWM. Anal Chem. 2007; 79:3048–3055. [PubMed: 17370998]
123. Zhang B, Galusha J, Shiozawa PG, Wang G, Bergren AJ, Jones RM, White RJ, Ervin EN, Cauley CC, White HS. Anal Chem. 2007; 79:4778–4787. [PubMed: 17550232]
124. Zhang B, Zhang Y, White HS. Anal Chem. 2006; 78:477–483. [PubMed: 16408930]
125. Wang C, Liu Q, Shao X, Yang G, Xue H, Hu X. Talanta. 2007; 71:178–185. [PubMed: 19071286]
126. Wang C, Hu X. Talanta. 2006; 68:1322–1328. [PubMed: 18970467]
127. Szamocki R, Velichko A, Holzapfel C, Mucklich F, Ravaine S, Garrigue P, Sojic N, Hempelmann R, Kuhn A. Anal Chem. 2007; 79:533–539. [PubMed: 17222017]
128. Pejcic B, De Marco R, Parkinson G. Analyst. 2006; 131:1079–1090. [PubMed: 17003853]
129. Rogers KR. Anal Chim Acta. 2006; 568:222–231. [PubMed: 17761264]
130. Wang J. Biosens Bioelectron. 2006; 21:1887–1892. [PubMed: 16330202]
131. Xu Z, Chen X, Dong S. TrAC, Trends Anal Chem. 2006; 25:899–908.
132. Castañeda MT, Alegret S, Merkoçi A. Electroanalysis. 2007; 19:743–753.
133. Zhao W, Xu J-J, Chen H-Y. Electroanalysis. 2006; 18:1737–1748.
134. Willner I, Zayats M. Angew Chem, Int Ed Engl. 2007; 46:6408–6418. [PubMed: 17600802]
135. Willner I, Baron R, Willner B. Biosens Bioelectron. 2007; 22:1841–1852. [PubMed: 17071070]
136. Asphahani F, Zhang M. Analyst. 2007; 132:835–841. [PubMed: 17710258]
137. Badihi-Mossberg M, Buchner V, Rishpon J. Electroanalysis. 2007; 19:2015–2028.
138. Koncki R. Anal Chim Acta. 2007; 599:7–15. [PubMed: 17765058]
139. Veetil JV, Ye K. Biotechnol Prog. 2007; 23:517–531. [PubMed: 17458980]
140. Ricci F, Volpe G, Micheli L, Palleschi G. Anal Chim Acta. 2007; 605:111–129. [PubMed: 18036374]
141. Beissenhirtz MK, Scheller FW, Viezzoli MS, Lisdat F. Anal Chem. 2006; 78:928–935. [PubMed: 16448070]
142. Stoica L, Ludwig R, Haltrich D, Gorton L. Anal Chem. 2006; 78:393–398. [PubMed: 16408919]
143. McMahon CP, Rocchitta G, Serra PA, Kirwan SM, Lowry JP, O'Neill RD. Anal Chem. 2006; 78:2352–2359. [PubMed: 16579619]
144. Masson J-F, Kranz C, Mizaikoff B. Anal Chem. 2007; 79:8531–8538. [PubMed: 17944438]
145. Lin C-L, Shih C-L, Chau L-K. Anal Chem. 2007; 79:3757–3763. [PubMed: 17417817]
146. Tian F, Llaudet E, Dale N. Anal Chem. 2007; 79:6760–6766. [PubMed: 17672522]
147. Yu B, Long N, Moussy Y, Moussy F. Biosens Bioelectron. 2006; 21:2275–2282. [PubMed: 16330201]
148. Han JH, Taylor JD, Kim DS, Kim YS, Kim YT, Cha GS, Nam H. Sens Actuators, B. 2007; 123:384–390.
149. Schoenfisch MH, Rothrock AR, Shin JH, Polizzi MA, Brinkley MF, Dobmeier KP. Biosens Bioelectron. 2006; 22:306–312. [PubMed: 16483759]
150. Musameh MM, Schauman NLM, Meyerhoff ME. Electroanalysis. 2006; 18:2043–2048.
151. Morales MD, Serra B, de Prada AG-V, Reviejo AJ, Pingarrón JM. Analyst. 2007; 132:572–578. [PubMed: 17525815]
152. Carelli D, Centonze D, Palermo C, Quinto M, Rotunno T. Biosens Bioelectron. 2007; 23:640–647. [PubMed: 17768043]

153. Liu G, Lin Y. Anal Chem. 2006; 78:835–843. [PubMed: 16448058]
154. Male KBH, Santini SJM, Luong JHT. Anal Chem. 2007; 79:7831–7837. [PubMed: 17874847]
155. Luo XK, Morrin AJA, Smyth MR. Anal Chim Acta. 2006; 575:39–44. [PubMed: 17723569]
156. Kurusu FT, Saito HA, Tomita A, Kadota A, Kayahara N, Karube I, Gotoh M. Analyst. 2006; 131:1292–1298. [PubMed: 17124536]
157. Qu S, Wang J, Kong J, Yang P, Chen G. Talanta. 2007; 71:1096–1102. [PubMed: 19071418]
158. Zhang Y, Zeng G-M, Tang L, Huang D-L, Jiang X-Y, Chen Y-N. Biosens Bioelectron. 2007; 22:2121–2126. [PubMed: 17081742]
159. Jena BK, Raj CR. Anal Chem. 2006; 78:6332–6339. [PubMed: 16970306]
160. Zhang H, Hu N. Biosens Bioelectron. 2007; 23:393–399. [PubMed: 17561388]
161. Xu S, Tu G, Peng B, Han X. Anal Chim Acta. 2006; 570:151–157. [PubMed: 17723393]
162. Shan D, Zhu M, Han E, Xue H, Cosnier S. Biosens Bioelectron. 2007; 23:648–654. [PubMed: 17768039]
163. Ricci F, Lai RY, Heeger AJ, Plaxco KW, Sumner JJ. Langmuir. 2007; 23:6827–6834. [PubMed: 17488132]
164. Arora K, Prabhakar N, Chand S, Malhotra BD. Biosens Bioelectron. 2007; 23:613–620. [PubMed: 17855071]
165. Wong ELS, Gooding JJ. Anal Chem. 2006; 78:2138–2144. [PubMed: 16579591]
166. Hansen JA, Mukhopadhyay R, Hansen JO, Gothelf KV. J Am Chem Soc. 2006; 128:3860–3861. [PubMed: 16551064]
167. Jenkins DM, Chami B, Kreuzer M, Presting G, Alvarez AM, Liaw BY. Anal Chem. 2006; 78:2314–2318. [PubMed: 16579614]
168. Gao Z, Agarwal A, Trigg AD, Singh N, Fang C, Tung C-H, Fan Y, Buddharaju KD, Kong J. Anal Chem. 2007; 79:3291–3297. [PubMed: 17407259]
169. Vermeeren V, Bijnens N, Wenmackers S, Daenen M, Haenen K, Williams OA, Ameloot M, vande Ven M, Wagner P, Michiels L. Langmuir. 2007; 23:13193–13202. [PubMed: 18004892]
170. Li A, Yang F, Ma Y, Yang X. Biosens Bioelectron. 2007; 22:1716–1722. [PubMed: 16959483]
171. Flechsig G-U, Reske T. Anal Chem. 2007; 79:2125–2130. [PubMed: 17326604]
172. Fanjul-Bolado P, Hernandez-Santos D, Gonzalez-Garcia MB, Costa-Garcia A. Anal Chem. 2007; 79:5272–5277. [PubMed: 17569504]
173. dos Santos Riccardi C, Yamanaka H, Josowicz M, Kowalik J, Mizaikoff B, Kranz C. Anal Chem. 2006; 78:1139–1145. [PubMed: 16478105]
174. Lubin AA, Lai RY, Baker BR, Heeger AJ, Plaxco KW. Anal Chem. 2006; 78:5671–5677. [PubMed: 16906710]
175. Li X, Lee JS, Kraatz H-B. Anal Chem. 2006; 78:6096–6101. [PubMed: 16944889]
176. Hvastkovs EG, Buttry SA. Anal Chem. 2007; 79:6922–6926. [PubMed: 17696404]
177. Miranda-Castro R, de-los-Santos-Alvarez P, Lobo-Castanon MJ, Miranda-Ordieres AJ, Tunon-Blanco P. Anal Chem. 2007; 79:4050–4055. [PubMed: 17477503]
178. Shrestha S, Yeung CMY, Mills CE, Lewington J, Tsang SC. Angew Chem, Int Ed. 2007; 46:3855–3859.
179. de-los-Santos-Alvarez N, Lobo-Castanon MJ, Miranda-Ordieres AJ, Tuñón-Blanco P. J Am Chem Soc. 2007; 129:3808–3809. [PubMed: 17355135]
180. Cheng AKH, Ge B, Yu H-Z. Anal Chem. 2007; 79:5158–5164. [PubMed: 17566977]
181. Hansen JA, Wang J, Kawde A-N, Xiang Y, Gothelf KV, Collins G. J Am Chem Soc. 2006; 128:2228–2229. [PubMed: 16478173]
182. Baker BR, Lai TY, Wood MS, Doctor WH, Heeger AJ, Plaxco KW. J Am Chem Soc. 2006; 128:3138–3139. [PubMed: 16522082]
183. Xiang Y, Xie M, Bash R, Chen JJL, Wang J. Angew Chem, Int Ed. 2007; 46:9054–9056.
184. Zhou L, Ou L-J, Chu X, Shen C-L, Yu R-Q. Anal Chem. 2007; 79:7492–7500. [PubMed: 17722881]
185. Zheng J, Feng W, Lin L, Zhang F, Cheng G, He P, Fang Y. Biosens Bioelectron. 2007; 23:341–347. [PubMed: 17583489]

186. Li X-M, Ju H-Q, Ding C-F, Zhang S-S. Anal Chim Acta. 2007; 582:158–163. [PubMed: 17386488]
187. Liu J, Su B, Lagger G, Tacchini P, Girault HH. Anal Chem. 2006; 78:6879–6884. [PubMed: 17007510]
188. Dong HL, Chen CMW, Zhou Q, Zeng ZX, Luong JHT. Anal Chem. 2006; 78:7424–7431. [PubMed: 17073408]
189. Ionescu REC, Herrmann SS, Marks RS. Anal Chem. 2007; 79:8662–8668. [PubMed: 17953450]
190. Wilson MS, Nie W. Anal Chem. 2006; 78:2507–2513. [PubMed: 16615757]
191. Zacco E, Pividori MI, Alegret S. Anal Chem. 2006; 78:1780–1788. [PubMed: 16536412]
192. Das J, Jo K, Lee JW, Yang H. Anal Chem. 2007; 79:2790–2796. [PubMed: 17311407]
193. Lu HK, Takkinen MPK, Guilbault GG. Biosens Bioelectron. 2007; 22:1756–1763. [PubMed: 16962310]
194. Viswanathan S, Wu L-C, Huang M-R, Ho JA. Anal Chem. 2006; 78:1115–1121. [PubMed: 16478102]
195. Tang D, Yuan R, Chai Y. Anal Chim Acta. 2006; 564:158–165.
196. Chumbimuni-Torres KY, Dai Z, Rubinova N, Xiang Y, Pretsch E, Wang J, Bakker E. J Am Chem Soc. 2006; 128:13676–13677. [PubMed: 17044681]
197. Chen Z-P, Peng Z-F, Luo Y, Qu B, Jiang J-H, Zhang X-B, Shen G-L, Yu R-Q. Biosens Bioelectron. 2007; 23:485–491. [PubMed: 17720472]
198. Wang J, Liu G, Engelhard MH, Lin Y. Anal Chem. 2006; 78:6974–6979. [PubMed: 17007523]
199. Ou C, Yuan R, Chai Y, Tang M, Chai R, He X. Anal Chim Acta. 2007; 603:205–213. [PubMed: 17963841]
200. Yu X, Munge B, Patel V, Jensen G, Bhirde A, Gong JD, Kim SN, Gillespie J, Gutkind JS, Papadimitrakopoulos F, Rusling JF. J Am Chem Soc. 2006; 128:1119–11205. [PubMed: 16433527]