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Chemical and biochemical sensors based on advances in materials chemistry

Mark E. Tess, James A. Cox *

Department of Chemistry and Biochemistry, Miami University, Oxford OH 45056, USA

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Abstract

The advance of materials chemistry has influenced the design of analytical sensors, especially those using spectroscopic or electrochemical methods for generating the signal. New methods of immobilizing enzymes, chromophores, and electron-transfer catalysts have resulted from initiatives in materials science. Systems based on sol-gel chemistry are especially noteworthy in this regard, but other important materials for chemical and biochemical sensors include zeolites, organic polymers, and various conducting composites. Applications cited include determinations of inorganic ions, gases, neurotransmitters, alcohols, carbohydrates, amino acids, proteins, and DNA.

Keywords: Review; Biosensors; Sensors; Sol-gel chemistry; Polymers; Carbon composites

1. Introduction

The development of stand-alone devices to monitor the concentration of selected chemical species in complex samples has been a goal in analytical science for several decades. As breakthroughs in areas such as micromachining, fiber optic technology, chemometrics, enzyme immobilization chemistry, and ultramicroelectrode design have appeared, sensor development has benefited. The recent emphasis on materials science has opened new approaches to analytical sensing. In this review, some important areas of overlap between sensor development and materials science will be described. Two areas dominate in this regard. First, certain materials have promise as means of encapsulating reagents in a manner that enhances stability during exposure to liquid samples. It will be illustrated that solids prepared by sol-gel chemistry shrink around hosted reagents, thereby blocking them from leaching into the sample solution, but possess a porous structure that provides access by small analytes. Second, materials with properties that are compatible with such interrogation methods as electrochemistry and spectroscopy are required for service as sensor platforms; advances in this area will be discussed as well.

Generally, this review will be restricted to cases where the bulk property of the material is exploited. In studies where the active material is in

* Corresponding author. Tel.: +1 513 5292813; fax: +1 513 5295715; e-mail: coxja@miavxl.muohio.edu

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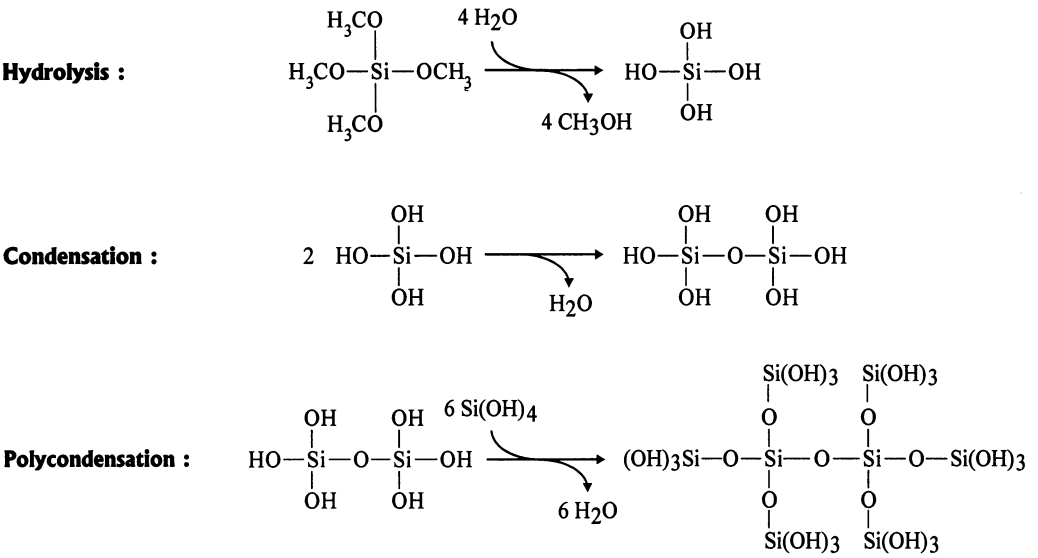

Fig. 1. Description of the sol-gel process. The process is the same with other alkoxide groups.

the form of a thin film, the distinction between bulk- and surface-modification is not clear; hence, a few examples of the latter will be cited. The focus of this review is on the signal-producing element; precluded is discussion of materials, such as fiber optics, that facilitate the spatial transfer of chemical and electronic information. Also not covered is the field of potentiometric ion-selective electrodes.

Primary focus will be on the following materials: solids prepared by sol-gel chemistry, carbon-based composites, and organic ionomers. Electrochemical and optical methods of signal generation are emphasized, but some examples employing other techniques, such as ion conductivity, will be cited.

2. Sensors based on materials prepared by sol-gel processes

Sol-gel science originated more than 150 years ago as a means of preparing ceramics. In recent years this area of chemistry has experienced renewed interest. When performed at low temperatures, porous solids are formed that can be interrogated by a variety of spectroscopic and electrochemical methods; hence, these materials are compatible with the major approaches for converting information from the chemical to the electrical domain. Moreover, sol-gel chemistry has been shown to provide a means of immobilizing enzymes in a manner that preserves their selectivity and activity and that allows their interaction with substrates originating in contacting liquid and gas phases [1–3]; hence, several reports of biosensors based on these materials have appeared recently.

The scope of sol-gel synthesis is too broad to present here. Perhaps the most detailed coverage is in a book by Brinker and Scherer [4], but many reviews contain useful summaries of the chemistry of these processes and of the characterization of the products [1,2,5]. Silica that is prepared by the hydrolysis of tetraalkyl orthosilicates is the most common of these materials for use in sensors. The general procedure involves a hydrolysis step that is followed by condensation as shown in Fig. 1. The product of the condensation reaction undergoes further reaction with the hydrolysis product to form the sol-gel network (polycondensation). These steps are catalyzed by the addition of an acid or a base.

A glass-like 'xerogel' is formed when the product is thoroughly dried. In practice, this term is applied to any gel that has been dried for several days, even at low temperatures; however, when the aging/drying is performed at or near room temperature, the solids can contain several percent (wt.) residual solvent. We have studied the solvent content (water and ethanol) in silica by thermogravimetric analysis [6]. After 3–7 days of drying, the weight percent of solvent was in the range of 25, 35 and 50% when the gel was aged at 9, 33 and 76% humidity, respectively. The residual solvent is primarily water, even with the process shown in Fig. 1. A study by Baddour et al. [7] on vanadia gels showed that the xerogel had a formula of $V_2O_5 \times 1.6\ H_2O$ when formed at room temperature. Wu et al. [8] recently addressed the nomenclature; they used the terms 'dried gel' to denote those that were made in a manner that did not remove all residual solvent (by fusion, for example) and 'aged gel' to describe those prepared to intentionally retain solvent. Here, the general term 'gel' is used when the distinction between xerogel and dried gel is not important.

Proteins are often denatured during immobilization; however, sol-gel chemistry yields a matrix that retains the protein's native conformation and reactivity. When prepared at or near room temperature, the dried gel matrix, although rigid, provides an aqueous environment inside the pores which host the dopant. As described by Ellerby et al. [9], the addition of a buffer after the hydrolysis of tetramethyl orthosilicate sol and prior to the gelation and the encapsulation of the protein, produced an environment that prevented denaturation and/or aggregation of proteins. This synthetic route allowed the physical entrapment of the biomolecule by allowing the sol-gel network to form around the dopant and thus provided a method to encapsulate the dopant that is almost independent of the dopant's size.

The use of sol-gel chemistry in the development of sensors has been reported extensively in the past few years. Optical sensing has dominated this field, but electrochemistry also has been employed. As shown below, 'proof-of-concept' reports rather than practical applications are dominant; however, because this field is rapidly emerging, it is included in the present review.

2.1. Optical sensors

Silica can be prepared by sol-gel chemistry to have pore diameters controlled within the several angstrom-to-nanometer range and to have transparency over a wide range of the UV-visible spectrum; hence, it is well-suited as a support for optical sensors. The initial research on such sensors has been reviewed by Lev [10] and by Dave et al. [2]. A typical example of an optical sensor is a silica monolith that contains immobilized *o*-phenanthroline; when contacted to a solution of Fe^{2+}, the characteristic color of the complex is formed in the monolith [11]. A sigmoidal calibration curve (absorbance versus concentration) is obtained with a detection limit below 100 ng l^{-1}. The leaching of the immobilized *o*-phenanthroline was reported as very slow; over a 10 day period, $< 0.3\%$ was leached upon immersion in aqueous solutions [12]. An early example of a biosensor is one for glucose which utilizes the co-immobilization of glucose oxidase (GOx), horseradish peroxidase (HRP) and quinoneimine dye precursors [13]. The hydrogen peroxide that is yielded by the GOx-catalyzed oxidation of glucose by dioxygen undergoes further reaction in the presence of HRP to form the dye. A selection of typical reports on optical sensors based on platforms prepared by sol-gel chemistry is summarized in Table 1. A summary of selected recent reports on the materials aspect of such platforms and of the results of selected, recent applications is presented below.

Table 1
Selected optical sensors employing sol-gel derived matrices

Analyte(s)	System	Ref.
Glucose	Glucose oxidase and a dye in silica	[1]
Metal ions	Chromophoric complexing agents in silica	[12]
pH	Fluoroscein-doped silica	[14]
Sulfate	Rhodizonate and BaF_2 co-encapsulated in silica	[15]
Pesticides	Sol-gel encapsulated cholinesterase; fluorescence	[16]
Dissolved O_2	Myoglobin in silica	[17]
NO, CO	Metalloproteins in silica	[18]

A demonstration of the importance of the porous nature of glassy zirconia and titania prepared by a sol-gel process was provided by Dulebohn et al. [19]. The solid hosted a Rh(I) complex that reacted reversibly with CO. The complexation resulted in changes in the visible and infrared spectra and in the electrochemical behavior of the complex, thereby providing a basis for sensing CO. Reversibility of the reaction was achieved by purging with N_2.

A major concern with the use of sol-gel materials as platforms for sensors is the response time that can be achieved. MacCraith et al. [20] addressed this and other technical issues in an apologia for employing thin films of these materials in sensors. An example presented was the development of an oxygen sensor based on the quenching of the fluorescence of a Ru(II) complex that was immobilized in a gel film (about 300 μm thickness) on a silica optical fiber. Curing at 73°C for 24 h provided a stable material with good optical properties and a response time of < 5 s. Another important point was that the film adhered well with the silica fiber.

As gels are aged, they undergo changes in their internal environment. Bright and co-workers have studied the aging of silica with rhodamine 6G and 1,3-bis(1-pyrenyl)propane (BPP) as fluorescent probes [21,22]. In the former report, two distinct microenvironments within aged silica were found. Aggregation of fluorophores and the perturbation of its flexibility by the shrinking matrix is avoided by selecting these compounds to have molecular sizes smaller than the pore diameters of the xerogels. Among the reasons for selecting BPP as a probe was that it is neutral and unlikely to interact with the gel matrix. In terms of optical sensing, an important finding was that the ageing of gels continues for at least one year, over which some instability is observed in the spectra of encapsulated materials [23]. When the active agents in optical sensors can interact with the gel matrix, the influence of aging on the spectral properties can be important and difficult to predict. For example, a blue-shift of the maximum emission wavelength of a molecule that is sensitive to the rigidity of the microenvironment occurs differently in mixed alumina–silica and in silica alone [24].

These factors suggest that it cannot be assumed that the photochemistry of a given system in solution is operative in gels, especially in those that are dried extensively. If the chromophore or fluorophore has molecular dimensions that are greater than the pore diameter and/or if they strongly interact with the gel backbone, an ageing effect on the spectroscopic behavior, and hence an unstable optical sensor, is predicted. These possible problems notwithstanding, many promising optical sensors that are based on platforms prepared by sol-gel chemistry have been reported.

In contrast to the above potential problems, it generally is considered that enzymes and other proteins that are immobilized by sol-gel processes retain the selectivity and activity of free solution. This is supported by a study of the Soret band of cyt *c* in a solution, an aged gel, a xerogel, and a rehydrated xerogel (rehydration does not change the pore diameter) [25]. Based upon the wavelength of maximum absorbance, the results are consistent with the hypothesis that the macromolecule acts to template the gel, thereby creating a pore environment that is not restrictive. Yet, the restricted access (essentially nanoporous and subnanoporous filtration) into the gel of species originating in contacting liquid phases contributes to the stabilization of these encapsulated enzymes. Hence, biosensing is an important application of sol-gel chemistry.

Among the recent applications is a sensor for atrazine [26]. Anti-atrazine monoclonal antibodies were encapsulated in silica that was prepared by hydrolysis of tetramethyl orthosilicate. The antibodies were added to the sol, and the gelation was performed under conditions that yielded a solid in a few minutes. The loaded gels were stored at 4°C until use (the best results were obtained with storage restricted to 2 days). Among the important results were that leaching of the antibody did not occur, binding of ng-quantities of antigen were observed, and interference by large quantities of unrelated protein in the sample did not occur.

The potential and the present limitations of optical biosensing with enzyme-loaded gels are discussed by Aylott et al. [27]. Here, a silica gel that contained periplasmic nitrate reductase (Nap)

was used to determine nitrate. The quantitation was based on measurement of a change in the UV-visible absorbance of the Nap upon exposure of the silica monolith to samples. To accommodate this large enzyme without precipitation in the sol, the water content was increased over that normally used in sol-gel chemistry. This increased the pore diameter of the gel; yet, leaching of the enzyme (mass, 109 kDa) was not observed. The encapsulated enzyme remained active for at least 6 months when the loaded gel was stored at 4°C in contact with phosphate buffer (pH 7.6). Prior to use, the Nap was reduced with sodium dithionite. This process must be monitored in order to assure that there is no excess chemical reductant in the gel. Upon contact with a nitrate sample, the absorbance of Nap changes at 550 nm. The calibration curve is linear from 0-1.5 μM nitrate with a detection limit of about 0.1 μM. The sensitivity varies from gel-to-gel because of variation in thickness.

In summary, it is apparent that the conversion of optical biosensing chemistry from homogeneous solution to gel-based systems can be achieved. It is necessary to avoid precipitation in the sol, which destroys the optical quality of the resulting gel, and to be cognizant of the requirement of the encapsulation chemistry to be compatible with maintaining the activity of the enzyme. The physical advantage of a loaded gel over solution chemistry may be offset by the times required for reagents to permeate the porous structure and for the baseline to be restored. This is compensated for in part by using disposable sensors and by using thin films rather than monoliths. Another problem with gels is the optical instability that results from ageing effects over a time scale of a year. This is one reason why there is considerable interest in finding other means of generating an analytical signal in gel-based sensors; here, amperometric methods are especially promising.

2.2. Amperometric sensors

Electrochemistry of solids prepared by sol-gel chemistry dates to the 1960's where efforts to develop reversible cathodes based upon conducting gels were initiated. A common system studied was vanadia in contact with propylene carbonate that contained lithium perchlorate; conversion of V^{V} to V^{IV} with concomitant intercalation of Li^{+} was the electrode reaction. Amperometric sensors that use gels as the electrolyte are a recent development. Here, four general systems have been employed. One is to use intrinsic conductors such as vanadia; a second is to use non-conductors such as silica but with the preparation procedure designed to retain a liquid electrolyte in the pores; the third is to use non-conductors in contact with a liquid electrolyte; and finally, several studies have used composites of non-conducting gels with conducting materials such as graphite powder to exploit the merits of gels as hosts for reagents without compromising the ability to use amperometric methods to quantify the samples. As in the case of optical systems, both biochemical and chemical sensors have been devised.

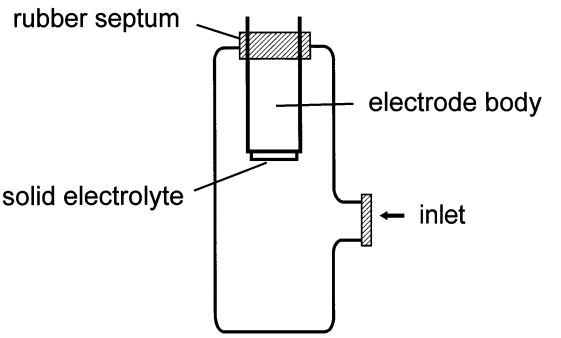

Fig. 2. Static gas cell for amperometric sensing. The inlet port can be used to inject gases directly or to introduce a liquid sample for head-space analysis.

Vanadia-based sensors have been used for the study of gaseous analytes. Because it is highly colored, it is not compatible with optical interrogation. We developed an amperometric sensor for ammonia which employed a three-electrode array that was coated with a thin film of vanadia as the active element [28]. Ammonia is not normally electroactive; however, by modifying the indicator electrode with a film of a ruthenium oxide catalyst prior to coating the array with vanadia, the oxidation of ammonia was promoted. Using a static gas sample (Fig. 2), a calibration curve that was linear over the range 2–27 μmol ml^{-1} (gas) was

obtained. We also developed a carbon monoxide sensor using silica that was formulated to retain water in the pores as the solid electrolyte [6]. The detection limit was 5 ppm (volume). However, the most important factors were that the sensitivity remained constant for at least 40 days after an initial drop over the first 2–4 days and the sensitivity was independent of humidity over the range 9–76%. This is in contrast with the humidity dependence of the sensitivity of comparable sensors but with organic polymers as the electrolyte. Dave et al. [29] reported a biosensor for detection of gaseous ethanol with hexacyanoferrate as the mediator and alcohol dehydrogenase as the enzyme. The presence of ethanol changed the $Fe^{III,II}$ ratio, thereby influencing the electron self-exchange current across the silica host. Gold electrodes contacted to the silica monolith completed the sensor element.

Gel-based amperometric sensors for liquid-phase analytes date to a report by Tatsu et al. [30] involving the flow injection determination of glucose in contact with gel-immobilized glucose oxidase. A linear response was observed over the range 0.2–4 g glucose l^{-1}. Glezer and Lev extended this approach into the development of a stand-alone sensor for glucose [31]. A calibration curve, albeit nonlinear, was reported over the range 1–14 mM glucose.

Collinson described a voltammetric sensor based on molecular imprinting an organically modified silicate by dopamine (Pittsburgh Conference, 1998, Abstract 401). Phenyltrimethoxysilane and tetramethoxysilane were used to form the silica gel around dopamine using the analogous reactions to those in Fig. 1. Leaching the dopamine from a film of the gel on an electrode surface yielded a voltammetric sensor with selectivity accrued in part by size exclusion. The response toward dopamine and related catecholamines was greater than toward ascorbic acid, DOPAC and L-DOPA.

The development of composites of gels with conducting materials promises to increase the practicality of these sensors for experiments where contact with solution is required. Lev and coworkers introduced a composite of silica and graphite that illustrated several potential merits of this material [32,33]. The powder filled the pores, thereby limiting shrinkage during drying and ageing. A possible outcome is less tendency to fracture when these monoliths are contacted with a liquid, but another attribute is that the rigid product is easily renewed as an electrode material by polishing. Second, a two-step procedure was developed for encapsulation of the enzyme; this minimized loss of activity by the environment present during conventional sol-gel chemistry. Third, the non-porosity of this composite protected the enzyme in the internal portion of the monolith from passivation by harsh samples; polishing exposed fresh enzyme after a given experiment. Tetrathiafulvalene and ferrocene were incorporated as mediators with glucose oxidase as the enzyme in a demonstration of use of this material as a glucose sensor. By changing the dopant, the function of the sensor was varied; for example the dopants, hydroquinone and Ag/AgCl, yielded sensors for pH and halides, respectively [32].

A new application of materials for sensors is to combine them with micromachining technology. This will make disposable sensors more attractive, and in addition, will provide new approaches to achieving selectivity. For example, Wang et al. [34] used screen-printed biostrips as sensors for glucose. Sol-gel chemistry was used as the means of immobilizing glucose oxidase, and dispersion of the loaded gel in carbon provided the 'ink'. But, in addition, a ruthenium oxide catalyst was dispersed in the composite to provide for the direct electrochemical determination of the hydrogen peroxide produced by the enzymatic reaction.

In summary, composites overcome some of the difficulties of using gels directly as sensors. Perhaps the most important point is the ability to block shrinkage, which, in turn, minimizes the problem of fracturing when introduced to solution. The loss of the porosity does eliminate some features of gels that are important; an example is the ability to function as a solid electrolyte for gas sensing. However, the response time and the time to return to a baseline signal are both enhanced. By using these composites, optical sensing is virtually precluded. As a result, the trend toward employing these materials may enhance research on the topic of amperometric sensors.

3. Polymers

Polymeric materials have played important roles in the development of sensors for their ability to increase analyte sensitivity and selectivity via phase-transfer chemistry. Sensors such as surface acoustic wave devices or electrochemical systems achieve practical sensitivity by coating these materials with polymers at which adsorption or absorption occur. These processes increase selectivity by discriminating against interferents. In addition, polymer films can be used to prevent fouling and to immobilize enzymes or chelators. When preconcentration does not occur, the linear range can be extended in some cases by decreasing the flux of the analyte, a process that lowers sensitivity.

Several techniques are available for applying polymer films; they include solvent casting, spin coating, and electropolymerization. Solvent casting is a procedure in which the dissolved polymer is coated onto the substrate, and the solvent is evaporated. These types of films do not form uniform, reproducible coatings. Spin coating allows both a controlled and uniform thickness on the substrate. Here the polymer solution is dropped on a rotating substrate, and the solvent is evaporated while spinning. Electropolymerization is the process in which a polymer forms via free radical or ionic polymerization.

Several recent reviews have appeared that address the topic of polymer coatings on amperometric devices [35–37]. Sensors based on solid polymer electrolytes (SPEs) utilize the polymer as a supporting electrolyte for use in nonconducting media or the gas phase. Often, these systems rely on a wicking system to moisten the polymer and/or to condition the SPE prior to analytical measurements. Table 2 lists some examples of the determination of gases with SPE-coated electrodes.

Nafion is the most common SPE. It is a perfluorinated polymer consisting of a tetrafluoroethylene backbone with perfluorinated vinyl ether side chains that terminate with sulfonic acid groups. The use of Nafion in amperometric sensors exploits its proton conductivity, water diffusivity, gas permeability (CO, CO_2, and O_2), and chemical and electrochemical inertness. Although Nafion is not crosslinked, it is a highly ordered structure consisting of sulfonate groups forming hydrophilic pockets/channels in the hydrophobic matrix. This, in part, leads to the known humidity-dependent response toward analytes such as CO at a Nafion-coated electrode [40,41]. Nafion is a hygroscopic polymer, so changes in the water content cause the clusters to swell [45]. This swelling alters the size and shape of these channels, thereby changing the proton diffusion, which is the charge-carrier mechanism, through the polymer.

We have reported on the use of Nafion as both an SPE and solid phase extractor for the determination of several neutral organic compounds in the gas phase [43]. An interdigitated microelectrode (IME) was coated with a ruthenium oxide catalyst. A Nafion overlayer was then cast over the modified IME. The electrode assembly was placed in a closed cell to control the humidity. In a typical test, a linear response to 200–1000 μl of methanol vapor in a 15 ml cell like that in Fig. 2 was obtained.

A detector for NO in physiological media was reported by Malinski and Taha [46]. Nafion was used to discriminate against anionic interferents such as NO_2^-. Electrodes were covered with a polymeric film consisting of monomeric tetrakis(3-methoxy-4-hydroxyphenyl)porphyrin with nickel as the central metal and a Nafion overlayer. The porphyrin was the electrocatalyst for the detection of NO. Detection limits of 20 nM for differential pulse voltammetry and 10 nM

Table 2
Selected gas-phase sensors employing solid organic polymer electrolytes (SPEs)

Analyte(s)	SPE	Ref.
CO_2	Nafion	[38]
NO_2	Polystyrene	[39]
CO	Nafion	[40,41]
Methanol	Ruthenium oxide and Ru impregnated membrane	[42]
Methanol, *N*-nitrosamines	Nafion	[43]
Ethanol	Nafion	[44]

for amperometry were obtained. The current for the oxidation of 1 μM NO increased only 1% in the presence of 20 μM NO_2^-; without the Nafion overlayer, the response was three times larger than that with NO only. The sensor was applied to measurement of release of NO from endothelial cells [46] and to the determination of NO in blood [47].

An amperometric sensor for glucose and lactose was constructed by Liu et al. [48] using a β-cyclodextrin polymer. Glucose oxidase, β-galactosidase and mutarotase were cross-linked in the polymer and ferrocene was included in the cavities in a host-guest relation. β-galactosidase was used to convert lactose to D-α-glucose and mutarotase was used to convert this product to D-β-glucose; ferrocene served as an electron-transfer mediator. The sensors retained 92 and 86% of their activity towards the target analytes after 1 and 2 months storage, respectively. The sensor response to other carbohydrates and various amino acids was negligible.

Holtz and Asher [49] fabricated biosensors from crystalline colloidal arrays polymerized in a solid matrix (PCCA). The PCCAs were doped with molecular recognition groups that bind an analyte selectively (such as crown ethers for metal ions) or with molecular-recognition agents that react specifically to a single analyte (enzymes). The binding caused the PCCA to swell, increasing the mean distance between the colloidal spheres and thus shifting the Bragg peak of the diffracted light to longer wavelengths. A sensor for glucose was produced by attaching glucose oxidase (GOx) to polystyrene spheres. Placing the GOx-modified PCCA into a glucose solution resulted in swelling by producing the reduced flavin (in GOx), which is anionic at pH 7. The oxidized flavin is uncharged at this pH. The gel produced a redshift of 8 nm within 30 min for a glucose concentrations of 10^{-12} M. Several problems were identified with the sensor. First, the response was dependent on the amount of dioxygen in the solution due to the oxidation of the flavin and thus loss of sensor response occurred. Second, the response was dependent on the ionic strength.

The detection of oxygen by photoluminescent quenching of platinum octaethylporphyrin was demonstrated by Lee and Okura [50]. The porphyrin-polymer film was excited at 535 nm, and the luminescence was monitored at 646 nm. Polyvinyl chloride, polystyrene and silicone polymer (GP-197) were mixed with the porphyrin using a cosolvent. The excitation and emission wavelengths with this system were reported as further apart than with other organic dyes for the detection of oxygen. A detection limit for the sensor was not given, but the sensor responded to concentrations of oxygen down to 3% by volume and was stable for 1 year.

Direct comparisons between organic polymers and sol-gel materials as supports for sensors have not been made. At present, it appears that the former may offer greater sensitivity, but the latter are more versatile as hosts for signal-promotion, species such as enzymes, and are more easily made into humidity-independent sensors. When hosting is not required, organic polymers also may be advantageous as gas sensors in terms of long-range stability, which can be up to a year with Nafion wetted by a wicking system to a reservoir.

4. Conducting composite electrodes

Composite electrodes have proven to be useful for bulk modification with modifiers such as enzymes and inorganic catalysts. These electrodes are formed by the combination of a powdered electronic conductor and a binding agent. Typically, a form of carbon is used to provide electrical conductivity, but metal powders are also suitable. Forms of carbon provide a greater potential range, -1.4 to $+1.3$ V versus SCE in aqueous solution, than metal conductors. A binding agent then is chosen based on the application of the electrode. In many cases an electron transfer mediator is incorporated, and the analytical signal is obtained by the scheme in Fig. 3. As shown in Fig. 3B, the presence of an analyte, which is not electroactive in the absence of the mediator, amplifies the oxidation current and attenuates the reduction current for the mediator in Fig. 3A.

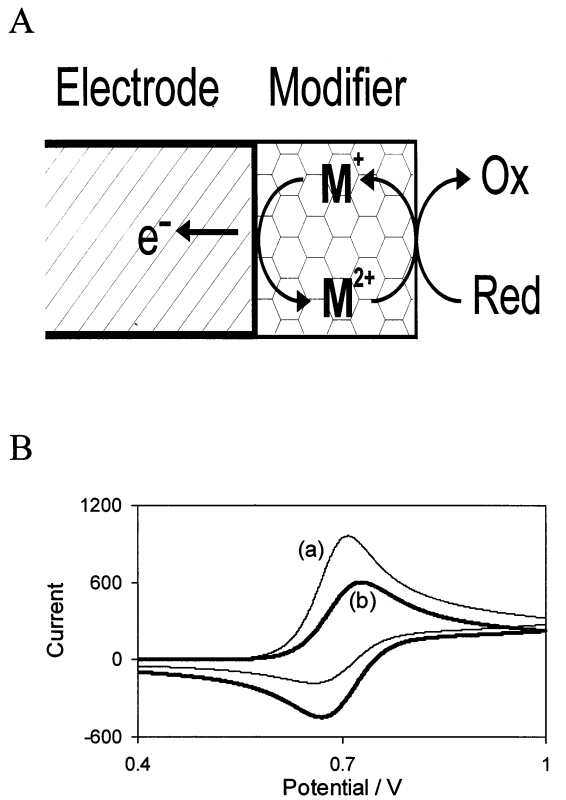

Fig. 3. Electron transfer mediation at a composite or surface-modified electrode. (A) Mediation scheme; (B) voltammetry behavior in the presence (a) and absence (b) of an analyte.

Composite electrodes can be fabricated using a soft, viscous binding agent such as mineral oil (carbon paste electrode, CPE) or a solid binding agent such as epoxy. The electrodes are non-porous, and, therefore, their surfaces can be renewed mechanically. Thus, the electrode surfaces can be restored reproducibly after fouling, modifier leaching, or other loss of catalytic activity. Composite electrodes can be prepared in a configuration of an array of microelectrodes. Here, micron-scale particles are dispersed in a manner that allows them to behave as geometrically independent electrode surfaces at the interface of the composite with the sample; yet, the packing density provides electrical conductivity. A detailed discussion of the merits of composite electrodes is presented by Tallman and Petersen [51].

4.1. Carbon paste electrodes

The CPE was first described by Adams [52]. The original intent was to produce a dropping, renewable carbon electrode; however, practical application was achieved only when the CPE was adapted to electrochemical detection in HPLC. The important developments that led to the use of CPEs as sensors as well as detectors for chromatography were bulk-modification by catalysts [53], including enzymes [54].

A CPE consists of a mixture of carbon powder and a pasting liquid. The pasting liquid is chosen to be inert, pure, and electroinactive and to have low volatility and solubility in the analyte solution. Paste materials include aliphatic hydrocarbons, mixtures of hydrocarbons, aromatic compounds, and silicone oils. The carbon is chosen to have a uniform particle size, high chemical purity, low adsorption of oxygen, and insignificant electrochemical impurities. The composite typically consists of approximately 70% carbon by weight [55]. A typical Nujol paste is prepared by mixing together 15 g of carbon and 9 ml of Nujol until the mixture is uniform. The modifier is placed either directly into the paste or absorbed to the carbon prior to mixing. Three recent reviews on CPEs provide a number of references on the type of carbon, binder and mediator used for various analytes [56–58]. After mixing, the paste is packed into an inert holder with electrical contact made to the back. A smooth CPE surface is prepared by skimming the assembly with a mild abrasive, such as an index card or weighing paper. This approach produces a working area with $< 5\%$ variation.

Recent reviews on CPEs have included bulk-modification with chemical and biological species [56–58]; hence, we will focus on properties that support their use in sensors, e.g. thermal stability of enzymes. Table 3 illustrates the range of analytes that have been studied at CPEs.

Wang et al. investigated the thermal stability of enzymes in CPEs at temperatures above 50°C [64]. The electrodes used carbon or 40% rhodium-on-carbon as the activity component and mineral oil or silicone grease as the binder. The stability of glucose oxidase (GOx), lactate oxidase (LOx),

alcohol oxidase (AOx), tyrosinase (polyphenol oxidase, PPO), peroxidase from horseradish (HRP), and L-amino acid oxidase (AAOx) were studied. In solution, GOx showed a 100% loss of activity after 4 h at 60°C. The loss after 2 h was 40% when entrapped in poly(phenylenediamine), and 5 and 15% losses were found when entrapped in a CPE after 10 and 120 days, respectively. At 80°C, the following lifetimes in carbon paste were obtained (enzyme, activity retained, time): PPO, 90%, 6 h; HRP, 50%, 6 days; AAOx, 50%, 12 h. A fragile enzyme, AOx, retained 70 and 30% activity after 1 and 5 days, respectively, at 60°C in a CPE, and LOx retained 40 and 15% activity over the same periods. The extended lifetime of the enzymes in carbon paste is consistent with results of investigations in hydrophobic solvents [65,66]. Also suggested was that enzyme unfolding is minimized in the rigid carbon paste environment [64].

In many cases, bulk-modified composites are developed after the successful demonstration of the utility of the analogous surface-modified system. Examples of surface-modified systems, which may be adapted into a composite in the future, are oligonucleotides, peptide nucleic acids (PNA) and DNA. These biological materials provide selectivity based on molecular recognition. The selective and sensitive detection for phenothiazine drugs using DNA as the modifier was reported with nM detection limits [67]. *Cryptosporidium* was determined using a 38-mer oligonucleotide unique to *Cryptosporidium* DNA [68]. Specific mutations of a p53 gene found in many types of cancer were identified using a PNA-modified electrode [69]. Hydrazine compounds were investigated using double-stranded (ds) DNA on the electrode surface [70]. In the last example, changes in the voltammetric response of the DNA-guanine peak (i.e. monitoring the loss of the guanine oxidation peak) provided the signal; calibration curves were linear for concentrations up to 4 ppb for 1,2-dimethylhydrazine. Above this concentration, the calibration curve was flat. The ds DNA was reproducibly coated on CPEs (RSD $< 6\%$, $n = 6$); selectivity was achieved for hydrazine derivatives over the parent (hydrazine) compound.

Table 3
Selected analytes sensed at carbon paste electrodes

Analyte(s)	Enzyme|mediator/catalyst[a]	Ref.
Glucose	Various enzymes[b]	[58]
Glucose	Glucose oxidase|ferrocene or rhodium	[59]
Amino acids	L- or D-Amino acid oxidase|iridium	[60]
Sugars	Oligosaccharide dehydrogenase|osmium	[61]
Phenols	Polyphenol oxidase	[62]
Cyanide	Asolectin, cytochrome c, cytochrome oxidase	[63]

[a] Mediator/catalyst used to lower redox potential of catalytically produced H_2O_2.
[b] Reference 58 cites over 50 biosensors for glucose determination using glucose oxidase, glucose NAD^+-dependent dehydrogenase, and glucose PQQ-dependent dehydrogenase enzymes.

4.2. Rigid composite electrodes

In contrast to carbon paste electrodes, rigid composite electrodes use a solidifying agent as the binder. These electrodes offer all of the merits of CPEs, but have an advantage over CPEs in that the modifier is not leached as readily into nonaqueous media. In addition, these electrodes allow use of a greater range of supporting electrolytes. Solidifying agents that have been employed include epoxy resin, Kel-F and Teflon. The last two are the more chemically inert.

Applications of polishable carbon-polymer biocomposites have been reviewed recently by Alegret [71]. Numerous references to the sensing of glucose, phenols, peroxides, alcohols, lactate, and pesticides are provided. Here, selected examples from the recent literature or from literature not cited by Alegret will be described, and some typical applications of rigid composite electrodes are compiled (Table 4). It should be noted that composite electrodes based on sol-gel chemistry are reviewed in a different section.

Our laboratory has developed an epoxy-based composite electrode that contains graphite powder which was modified with an electropolymerized ruthenium oxide catalyst [78]. The catalytic activity of the electrode was verified using As(III)

Table 4
Selected analytes sensed at rigid composite electrodes

Analyte(s)	Composite material	Modifier(s)[a]	Ref.
Glucose	Graphite-epoxy	Gox	[72]
Glucose	Graphite-epoxy	GOx/Au	[73]
Organic peroxides	Graphite-epoxy	HRP	[74]
Alcohols	Graphite-epoxy	YAD	[75]
Phenols	Graphite-epoxy	Tyrosinase	[76]
Peroxides	Graphite-Teflon	Peroxidase/ferrocyanide	[77]

[a] GOx, glucose oxidase; HRP, horseradish peroxidase; YAD, yeast alcohol dehydrogenase; second item listed was used to catalyze the reduction of catalytically produced H_2O_2.

as the analyte. The catalytic activity was retained for more than 1 year when stored in acidic solution or in the air-dried state. The relative standard deviation of 19 replicates of the determination of As(III) was 3% when the electrode was polished with 400- and 1200-grit carbide paper between trials. Here, it is important to note that As(III) is not electroactive when the catalyst is not present, so these data illustrate the reproducibility of the catalytic activity, not just the surface area. Based on previous work with analogous surface-modified systems, compounds such as thiocyanate [79,80], amino acids [79,81–83], insulin [84], and heparin [85] are amenable to sensing with this composite.

This ruthenium oxide composite was also active in micellar media [78]. Although the sensitivity toward *N*-nitrosamines was decreased by 50% relative to that in a simple electrolyte, the ability to use micellar media extends the range of applicability to high molecular-weight biomolecules. Pingarrón and co-workers have also reported the use of composite electrodes in micellar media [77]; the composite electrode (Teflon-graphite) was prepared by immobilizing horseradish peroxidase as a catalyst and potassium ferrocyanide as a mediator for the determination of peroxides.

Overall, carbon-based composites have been explored primarily as detectors for liquid chromatography and flow injection amperometry. But as shown above, they possess the characteristics required for stand-alone sensors, especially for applications requiring enzymes.

5. Miscellaneous

The trend toward miniaturization in analytical chemistry complicates the distinction between bulk materials and surfaces for sensors. Moreover, certain materials of importance do not fit well with the major categories of this review. In this section, some examples that illustrate these areas of overlap between materials chemistry and sensor development are presented.

Self-assembled bilayer lipid membranes (BLMs) as surface films provide a sensing element that is actually based upon their bulk properties. Nikolelis and co-workers have used modification of BLMs to develop sensors for a variety of species based upon changes in their conductivities when exposed to the analytes [86–88]. Incorporation of hemoglobin resulted in a sensor for CO_2 [86]. The BLM was stable for over 48 h. A calibration curve was linear over the range of 38–566 nM CO_2. Species that compete with CO_2 in terms of binding to hemoglobin interfered, but it was not severe. For example, 15 nM CO decreased the response toward 200 nM CO_2 by only 5%, and CN^- interference was comparable in magnitude. The general concept was used to sense DNA at the ng ml^{-1} level [87] and CN^- down to 4.9 nM [88].

Self-assembled monolayers (SAMs) have been shown to dramatically reduce the background current and 'undesirable' Faradaic currents in a glucose sensor [89]. The former was achieved by decreasing the double-layer capacitance, and the

latter, by attenuating the flux of interfering electroactive species to the electrode surface. The sensors were prepared by forming SAMs of 6-mercaptohexanol or 11-mercaptoundecanol on gold electrodes, coating with glucose oxidase, and cross-linking the glucose oxidase film with glutaric dialdehyde. The SAM also decreased the oxidation current of hydrogen peroxide formed by the enzymatic reaction. To circumvent this problem, water soluble ferrocene derivatives were used to shuttle charge between the enzyme layer and electrode surface. Other applications of SAMs have been recently reviewed [90].

An amperometric biosensor for phenol vapor was described which utilized a glycerol-based gel over an interdigitated electrode array as the reaction medium [91]. The gel was loaded with polyphenol oxidase to promote the conversion of the analyte to quinone. The sensitivity and selectivity were enhanced by the high solubility of phenol in glycerol, which allowed preconcentration to occur. A detection limit of 29 ppb (volume) at 40% relative humidity was reported. Linear calibration curves were obtained over the range of 1–12 ppm (volume), but the slope was humidity dependent. Interference from vapors of common organic solvents was not seen.

Zeolites are a class of aluminosilicates that have been studied for over 200 years. The properties of zeolites have led to applications as sorbents for gases and liquids, catalysts (especially for cracking of petroleum chemicals), cation exchangers, and molecular sieves based on shape-selection or size-exclusion. These properties has lead to the development of numerous modified electrodes [92–94]. One notable example is the preparation of zeolite-modified CPEs described by Wang and Walcarius [95]. Zeolite Y was used for the selective uptake of dopamine over ascorbic acid, which is important in that the oxidation potentials of these species are virtually identical. At $pH > 6$, dopamine is cationic and ascorbic acid is anionic; therefore, dopamine is selectively sorbed by zeolite Y. A CPE modified with 10 wt.% zeolite showed four and nine fold peak enhancements of dopamine for 1 and 4 min preconcentrations, respectively, over bare CPEs. Among the other applications of zeolites to sensors was the use of these materials as coatings on surface acoustic wave devices [96]; here, the detection of small organic gases was enhanced by the large surface area and molecular sieving of the zeolite.

Prussian blue (PB) films as solid electrolytes are being studied extensively as electrocatalysts. Because these mixed-valent films are ionic, not electronic, conductors, they do not short-circuit the electrode arrays over which they are deposited. McCormac et al. [97] illustrate the use of PB as a solid electrolyte in a sensor for methanol vapor. Because PB catalyzes the oxidation of many species, selectivity is a limiting factor of this approach.

Several other mixed-valent metal systems are being used as electrocatalysts in sensor development. As in the case of PB, the electron transfer reaction with the analyte is promoted by mediation (Fig. 3); hence, the process is not selective. Examples of materials suited to promote reductions are polyoxometalates, WO_3 and related metal oxides, and $VOSO_4$ and similar salts; a detailed discussion of these materials is in a recent review [37]. A promising material based on these compounds is to incorporate highly dispersed Pt (or other Pt group metal) microclusters within them. For example, Kulesza et al. [98] doped mixed-valent tungsten oxide with Pt and improved the reactivity toward gaseous O_2 and H_2O_2. Also, dispersions of Pt in organic polymers are well-known to provide an electrocatalytic material [99] (and references therein). But the use of general redox catalysts exacerbates the serious limitation of selectivity for applications to sensors.

6. Conclusion and projections

Sensor development and materials chemistry often are intertwined. Despite the great body of research literature on sensors, few practical, commercial systems (other than a number of potentiometric ion-selective electrodes) have emerged to date. Historically, selectivity has been the primary limitation. Enzyme-based biosensors provide the needed selectivity, but long-term stability and shelf life are practical limitations. Recent ad-

vances in materials science promise to alleviate these problems. Sol-gel chemistry, as one example, not only has promise as a means of addressing the limitations of enzyme-based systems but also is a route to hosting chemical reagents that can impart selectivity to the system. Composites, in general, are providing routes to achieving these characteristics.

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