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A review on ormosil-based biomaterials and their applications in sensor design

P. C. PANDEY
Department of Chemistry, Banaras Hindu University, Varanasi 221 005, India
email, p_c_pandey@satyam net.in

Abstract

The chemistry of sol–gel glasses is reviewed Sol–gel glasses derived from various sol–gel precursors are discussed. Alkoxide monomers containing various organic functional groups, e g. amino-, epoxy-, glycidoxy-, hydroxyl-, etc. leading to organically modified sol–gel glass (ormosil) are demonstrated. The development of composite sol–gel glasses derived from metal–ceramic complex is also reviewed. These ormosils behave as good biomaterial, which retain biological activity even after encapsulating the same during sol–gel processing. The application of such materials in sensor design is reviewed. Various parameters such as water contact angle and wetted surface area of ormosil affecting the performance of ormosil-based sensors are also discussed. Special attention has been paid to encapsulation/linkage of ferrocene derivatives within ormosil The electrochemistry of ferrocene encapsulated within the palladium–ormosil material is reviewed.

Keywords: Ormosil, alkoxide monomers, ferrocene, electrochemistry.

1. Introduction

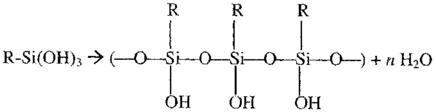
During the last two centuries, developments in ceramic chemistry have triggered several domestic and technological applications. Major limitation of conventional ceramic material has been high temperature needed during processing and the limitation of technology in forming desired complex geometrical configurations of the material. Recent development in sol–gel technology has now permitted the formation of ceramic material in desired shapes at low temperature. The main objective of this review is to provide a deeper insight into production and application of these new materials.

Early investigators observed that the hydrolysis of tetraethylorthosilicate (TEOS), [$Si(OC_2H_5)_4$] under acidic conditions yielded SiO_2 in several forms, e.g. a glass-like material in different geometrical forms. However, extremely long drying time required for their formation and fracture problems made the processes of limited technological application. The large volume of descriptive literature from chemists/chemical engineers/ceramic engineers/physicists of the period reveals sparse understanding of physicochemical principles governing such processes. During the dawn of the 20th century chemists got interested in the phenomenon of Liesegang rings through sol–gel process. The phenomenon of periodic precipitation and crystal growth from gels was investigated in detail. During the 1950s and '70s, Roy and his coworkers[1] recognized the potential of sol–gel process for achieving very high level of chemical homogeneity. They were able to synthesize a large number of novel ceramic oxide compositions involving Al, Si, Ti, Zn, etc. which could not be produced by traditional ceramic powder technology. The pioneering work of Iler's[2] in silica chemistry during this period led to commercial development of colloidal silica powders.[3] This concept has led to the production of a wide variety of composites with controlled morphologies and particle size.[3-4] The materials include hydroxides, sulphides and mixed phases and coated particles. While the ceramic powder technology encounters severe limitations due to agglomeration and high-temperature requirements, the sol–gel process yielded materials of high purity and homogeneity at low temperature. This process permits control of the surface and interfaces of materials during early stage of production.[5-6] A quantitative comparison on the merits of sol–gel-derived silica optics over the alternative high-temperature processing methods has been reported by Orcel *et al.*[7] The demonstration of potentially practical routes for production of new materials with unique properties coincided with the growing recognition that powder processing of materials had inherent limitation in homogeneity due to difficulty in controlling agglomeration.[6] The introduction of various functional groups into organic alkoxide has led to organically modified sol–gel glasses known as ormosil. This technology has provided deeper insight into macro-molecular organization of such materials and their effects on the properties of sol–gel glasses. The objective of this review is to focus on the chemistry of ormosil and its applications to sensor design.

2. Chemistry of sol–gel process

The sol–gel process, as the name implies, involves the production of inorganic matrices through the formation of colloidal suspension (sol) and gelation of the sol to form a wet gel (a globally connected solid matrix) which after drying forms the 'dry gel' state (xerogel). Three approaches are used for the production of sol–gel monoliths: 1. Gelation of a solution of colloidal powders, 2. Hydrolysis and polycondensation of alkoxide or nitrate precursors followed by hypercritical drying of gels, 3. Hydrolysis and polycondensation of alkoxide precursors followed by ageing and drying under ambient atmospheres. The chemistry of such a process can be explained from the following chemical equations:

$$\text{R-Si(OMe)}_3 + 3\,\text{H}_2\text{O} \rightarrow \text{RSi(OH)}_3 + 3\,\text{MeOH} \tag{1}$$



$$\text{R-Si(OH)}_3 \rightarrow (\text{—O—}\underset{\text{OH}}{\overset{\text{R}}{\text{Si}}}\text{—O—}\underset{\text{OH}}{\overset{\text{R}}{\text{Si}}}\text{—O—}\underset{\text{OH}}{\overset{\text{R}}{\text{Si}}}\text{—O—}) + n\,\text{H}_2\text{O} \tag{2}$$

Sols are dispersion of colloidal particles in a liquid. Colloids are solid particles with diameters of 1–100 nm. A gel is a interconnected, rigid network with pores of submicrometer dimensions and polymeric chains whose average length is greater than a micrometer. The term 'gel' embraces a diversity of combinations of substances that can be classified into four categories: (1) well-ordered lamellar structures, (2) covalent polymeric networks, completely disordered, (3) polymer networks formed through physical aggregation, predominantly disordered, and (4) particular disordered structures. When the pore of the liquid is removed at or near ambient pressure by thermal evaporation, drying shrinkage occurs and the monolith is termed xerogel. If the pore liquid is primarily alcohol based the monolith is often termed an alcogel. The generic term gel usually applies to either xerogels or alcogels, whereas aerogels are usually designated as such. A gel is defined as dried when the physically adsorbed water is evacuated. This occurs between 100° and 180°C. Heat treatment of a gel at elevated temperature substantially reduces the number of pores and their connectivity due to viscous-phase sintering. This is termed as densification. The density of the monolith increases and the porosity decreases during sintering. The porous gel is transformed to a dense glass when all pores are eliminated. Densification is completed at 1250–1500°C for gels made by method (1) and as low as 1000°C for gels made by method (3). The densification temperature decreases as the pore radius decreases and surface area of the gels increases. The density of silica glass made by densification of porous silica gel is amorphous whereas it is nearly equivalent in structure to vitreous silica made by fusing quartz crystals or sintering of SiO_2 powders made by chemical vapor deposition (CVD) of $SiCl_4$. The steps involved in making sol–gel-derived silica monoliths for methods (1–3) are associated to seven steps.[5, 7] These seven steps are: (1) mixing, (2) hydrolysis, (3) condensation, (4) ageing, (5) drying, (6) stabilization, and (7) densification.

Processes such as ageing, drying, stabilization and densification depend upon the gel structure. Since it is the relative rates of hydrolysis and condensation that determine the structure of the gel, it is essential to understand the kinetics of the hydrolysis (K_H) and condensation reaction (K_C), and the ratio of their rate constants (K_H/K_C). Additionally, many other factors including many species that are present in the solution influence the kinetics of hydrolysis and condensation. Furthermore, hydrolysis and polycondensation occur simultaneously. The variables of major importance are temperature, condensation of electrolyte (acid, base), nature of the solvent and the type of alkoxide precursor. Orcel *et al.*[7] observe that pressure also influences the gelation process. The kinetics of hydrolysis (K_H) increases with pressure; on the other hand, pressure is usually not a processing variable. In fact, many studies have reported the variation of gelation time, viscosity or textural characteristic (specific surface area) of the gel as a function of experimental conditions[8–10] without alternation in K_H or K_C. The influence of electrolyte concentrations on the hydrolysis of TEOS in different solvents showed that K_H increases linearly with the concentration of H^+ or H_3O^+ in acidic media and with the concentration of OH^- in basic medium. The nature of alkoxy group on the silicon atom also influences the rate constants. In general, the longer and the bulkier the alkoxy group the slower is the rate constant of hydrolysis (K_H).[9] The NMR spectra of silicon was reported to investigate the condensation of aqueous silicates at high pH. Hydrolysis and poly-condensation reactions initiate numerous reactive sites when mixing of trimethoxysilane (TMOS) and water occurs. The size of the sol particles and the crosslinking within the particles (i.e. density) depend upon the pH and R ratio where R is $[H_2O]/[Si(OR)_4]$. A number of investigators have observed that the time of gelation changes significantly with the sol–gel chemistry. Many investigators developed a method to measure the viscoelastic response of the gel as a function of shear rate. Gelation times (t_g) is not an intrinsic property of the sol and depends on the size of the container. The dependence of t_g on solution pH has not been fully determined. However, it appears from the work of Yamane *et al.*[11] that the curve t_g vs pH has a bell shape. In other words, gelation[11] can be nearly instantaneous for very acidic or basic solution of metal alkoxides. This behavior is very different from the gels prepared by destabilization of a silica sol where the curve has an S shape with maximum around the isoelectric point of silica (pH ~ 2) and a minimum near pH 5–6. The anion and solvent also play a role in the kinetics of gelation which can be either acid or base catalyzed. The longer and the larger the solvent molecule, the longer is the gelation times. Similarly, it has been shown that the longer and the larger the alkoxy group the longer is t_g. The amount of water for hydrolysis of alkoxysilane has a dramatic influence on gelation time.[12] When gelation process was allowed to occur at 70°C in the presence of HF as catalyst if $R = 2$, t_g is about 7 h which decreases to 10 minutes if $R = 8$. For low water content, generally an increase in the amount of hydrolysis water decreases the gelation time, although there is a dilution effect as well. It can be predicted that for higher water content, the gelation time increases with the quantity of water. Further viscosity of sol–gel precursors significantly influences the physical forms, e.g. fiber coating and monolith[13–20] of casted gel.

3. Sol–gel structure

The characterization of sol–gel geometry is important requirement and a few methods are available for nanometer scale of sol–gel glass. These are small-angle X-ray scattering (SAXS), neutron scattering (SANS) and light scattering (SALS), each of them giving complementary information and transmission electron microscopy of sol–gel geometry. SAXS allows the determination of a characteristic length of the particle (Guiniers radius of gyration or electronic radius of gyration) and a fractal dimension, which gives some information on the structure of the polymer (branched vs linear) and on the growth mechanism. The application of SAXS to a number of gel systems has been reported by various authors. SANS has also been applied to the study of silica sols.[21] Results similar to those from SAXS are obtained, but further development of the SANS technique may produce additional insight into the sol–gel process. SALS has received very little attention in the sol–gel literature. However, the characteristic dimension probed by visible light scattering is 710 nm, and therefore it cannot be used to characterize the early stage of the gelation process. Recent development of short-wave-length UV lasers may make it possible to extend light-scattering studies to 3 nm and thereby could follow most of the gelation process.

The major conclusion of the various scattering studies on the structure of sol–gel glasses is that acid-catalyzed sols develop a linear structure with very little branching. In contrast, base-catalyzed systems are characterized by highly ramified structures. Classical theory,[22] percolation theory[23–24] and fractal theory[25–26] are reported which are associated to the mechanistic behavior of the sol–gel gelation. The drying behavior of porous solids has been extensively studied by several workers.[27–30] However, most of the data have been on powder systems with relatively large pores. Ageing of a gel, also called syneresis, involves maintaining the cast object for period of time, hours to days, completely immersed in liquid. During ageing, polycondensation continues along with localized solution and reprecipitation of the gel network, which increase the thickness of inter-particle necks and decrease the porosity. An aged gel must develop sufficient strength to resist cracking during drying. Four processes, polycondensation, synerisis, coarsening, and phase transformation, can occur, singly or simultaneously. Extensive attempts have been made to describe ageing phenomena theoretically. During drying the liquid is removed from the interconnected pore network. Large capillary stresses can develop during drying when pores are small (220 nm). These stresses will cause the gels to crack catastrophically unless the drying process is controlled by decreasing the liquid surface energy. Additionally, cracking in gel structure can also be controlled by the addition of surfactants or elimination of very small pores by hypercritical evaporation which avoids the solid–

liquid interface or by obtaining mono-disperse pore size by controlling the rates of hydrolysis and condensation. After drying, the aerogel has a very low density and has a very good thermal insulation when sandwiched between evacuated glass plates.

4. Organically modified sol–gel glasses

Organic modifications[31-40] in sol–gel precursor provide better way of controlling nanoporous geometry of sol–gel glasses suitable for sensor design. Such precursor after following the sol–gel process leads to the formation of organically modified sol–gel glass (ormosil). Organic functionality during sol–gel processing could be used to increase the degree of crosslinking, improve film adhesion to its support, reduce the concentration of surface silanol group and to introduce reactive functional group that can be subsequently used for anchoring of molecular recognition species of a prepared xerogels. The sol–gel process provides a convenient method for the production of organically modified surface by incorporating alkoxysilane monomers that contain desirable functional group in the starting polymerization mixture.[35]

Ormosils (organically modified silica) and ormocers (organically modified ceramic) can be tailored from commercially available organo-functional alkoxysilanes. The ratio of tetra alkoxysilane and organotrialkoxysilane can be used to control the cation exchange capacity and polarity of the porous surface. Cyanoalkyltrialkoxysilane or chloroalkyltrialkoxysilane monomers can be used either alone or in combination with tetraalkoxysilanes to acquire controlled surface polarity and reduced ion exchange capacity. Monomers containing an Si–C bond and easily derivatised radical such as an amino, vinyl, epoxy or mercapto group can be used to prepare readily derivatised xerogels. These can be subsequently used as covalent anchors for specific chelating agents, redox mediator, or photometric reagents. A mixture of organically modified silica and other metal oxides can be used to adjust the hydrophobicity around metal oxides that do not form stable metal–carbon bonds. Additionally, the lower degree of cross-linking provides an inherent strain relaxation pathway allowing thick (>1 μm) silica film to be prepared in one coating step.[36] The high molecular weight silica oligomers are more stable than smaller ones, which provide excellent condition for necrophilic growth of large particles by feeding on low molecular weight-dissociated fragments.

5. Ormosil-based biosensors

Several reports on sol–gel glasses-based biosensors are available.[41–58] One of the potent applications of such materials is in the development of sensors particularly for attaching the sensing material to the surface of physicochemical transducers. A number of publications are available on the applications of sol–gel glass for the development of optical and electrochemical sensors.[37] Wang and Pamidi[56] developed biogel-based carbon inks that display compatibility with the screen-printing device to develop microband electrodes.

The development of electrochemical biosensor involves the coupling of biological components with polarizable or nonpolarizable electrodes. The use of sol–gel glass for the development of electrochemical biosensors has received great attention because of its possible commercial application. The development of such biosensors based on sol–gel glass is currently restricted mainly due to two major problems: 1) the requirement of controlled gelation of soluble sol–gel components at ambient conditions, and 2) preparation of sol–gel glass of smooth surface, controlled thickness and porosity. Additionally, the stability of biological element within the sol–gel network is another need to develop such sensors on commercial scale. Apparently, the synthesis of suitable biocompatible sol–gel glass of desired thickness and porosity is of considerable interest. The soluble materials leading to the formation of sol–gel glasses are the derivatives of alkoxysilane. These alkoxysilanes in acidic and some time in basic medium generate a solid network which in physical structure can be comparable to conventional glass. However, research is needed to synthesize such sol–gel glasses suitable for better performance as sensors and reactors of practical significance. The application of these glasses in sensor designing requires control synthesis of solid-state network with desired porosity and thickness. Additionally, the availability of suitable group within the solid-state network provides an advantage for the crosslinking of the sensing element to the network.

Zink and coworkers[33, 34] extensively studied silicate glasses obtained by the sol–gel method, which can provide such a host matrix that biomolecules immobilized by this method retain their functional characteristics to a large extent.[33, 34] They also reviewed more than 35 types of biologically derived encapsulated ceramic materials. Further studies by Zink and coworkers[48] demonstrated the biomolecular materials based on sol–gel-encapsulated proteins.

The sol–gel glass-modified electrodes prepared by introducing graphite particles (of size 1-2 μm) have received great attention.[51] The dispersed carbon provides the electrical conductivity essential for electrochemical measurements. These carbon–ceramic electrodes (CCEs) can be bulk-modified by organic, inorganic or biochemical species, which can subsequently be used in the preparation of Pd-modified, enzyme-doped carbon–ormosil composite material (organically modified sol–gel glasses). This made possible to cast silica–carbon matrices in virtually any desired geometrical configuration, including flat layers spread on insulating or conductive matrices, monolithic disks or rods or even in the form of miniature CCEs.[51] Another way for the construction of modified CCEs, which can be used for the large surface area amplification, is the use of hydrophilic and -phobic silica-forming monomers, such as cyanoethyltrialkoxysilane as hydrophilic monomer and methyltrimethoxysilane as hydrophobic monomer. When hydrophobic silica-forming monomers are used, the resulting electrodes reject water, leaving only segregated islands of carbon at the outermost surface in contact with electrolyte.[51] Thus, the ratio of hydrophilic and -phobic monomers in organically modified sol–gel CCEs is crucial in sensor design. Additionally, monomers containing an Si–C bond and an easily derivatised radicals through crosslinking such as an amino, vinyl, epoxy or mercapto groups can be used to prepare readily derivatised xerogels[37, 51, 55] and provide a convenient method for the production of organically modified surface which can be used as covalent anchor for specific chelating agents. Lower degree of crosslinking is an inherent strain relaxation pathway allowing thick (>1 μm) silica films to be prepared in one coating step.[21]

The reactive area of ormosil as determined by the term 'wettability' (wetted section of sol–gel glass) can also be increased by incorporating readily leachable, water-soluble components in matrix and dissolving them out by immersing the electrodes in an electrolyte solution. This leads wide-open channels for the penetrating electrolyte, thereby increasing the wetted section inside the sol–gel matrix. A typical example is the use of polyethyleneglycol.[51]

5.1. *Ormosil-based nonmediated biosensors*

The amperometric biosensor which does not require the participation of redox molecules having reversible electrochemistry is referred to as nonmediated biosensor whereas the participation of redox mediator in signal transduction generates a category of mediated biosensors.[59-63] We have developed ormosil using two organosilane precursors, i.e. 3-aminopropyl-triethoxy silane (relatively hydrophilic precursor) and 2-(3, 4-epoxycyclohexyl)-ethyltrime-thoxy silane (relatively hydrophobic precursor). The physical characteristics of sol–gel glass made from the two silanes[64] in the absence of polyethylene glycol and graphite powder were studied in the presence and absence of glucose oxidase (GOD). These two silanes result in a very smooth surface of sol–gel glass prepared by following a very simple one-step gelation process as compared to those prepared by following complex protocol of gelation, which requires sonication for the homogenization of monomers, and additive suspension reported by earlier workers.[50-53] A smooth sol–gel film without cracking and having a better performance when used as sensor was obtained using optimum concentrations of sol–gel precursors reported in the experimental section. These two silane precursors were used to develop four different systems of glucose oxidase encapsulated/sandwiched ormosil.[64, 65] Scanning electron microscopy having water-soluble additives (polyethylene glycol) evidently shows better porosity with smooth surface and evidently better sensing performance as discussed vide infra.

The organically modified sol–gel glass matrix inhibits intermolecular interactions of the encapsulated macromolecules. The presence of the rigid silicate cage prevents protein movement and the matrix functions as a solid solution of encapsulated biomolecules. First, we studied the electrochemical behavior of ormosil-based biosensors of four different compositions[64] in the absence of any electron-transfer mediator. The cyclic voltammetric studies were conducted between 0 and 1 V vs Ag/AgCl, whereas amperometric measurements were made at 0.70 V vs. Ag/AgCl. The cyclic voltammetry results for System-4, which was developed using 3-aminopropyltriethoxy silane, 2-(3, 4-epoxycyclohexyl)-ethyltrimethoxy silane, polyethylene glycol, glucose oxidase and graphite particles (1-2 μ), in the presence and absence of 50 mM glucose are shown in Fig. 1 between 0 and 1 V vs Ag/AgCl at the scan rate of 5 mV/s in 0.1 M

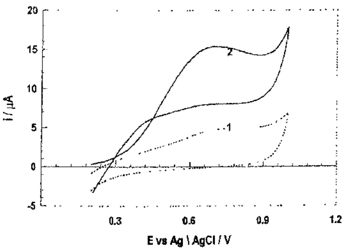


FIG. 1. Cyclic voltammograms of sol–gel-glass-modified glucose biosensor made from System-4 in the absence (1) and presence (2) of 50 mM glucose in 0.1-M phosphate buffer (pH 7.0) at the scan rate of 5 mV/s.

phosphate buffer pH 7.0 at 25^{0}C. Curve 1 shows voltammogram in the absence of glucose and Curve 2 in the presence of glucose. On the addition of glucose, large increase in anodic current corresponding to the oxidation of hydrogen peroxide was observed. The typical amperometric response using ormosil-based glucose biosensor was also studied (Fig. 2). The magnitude of anodic current in System-4 was greater than that observed in Systems-1–3. Inset (I) to Fig. 2 shows calibration curves of glucose analysis. System-1 shows the lowest amperometric response, whereas System-4 shows relatively larger amperometric response.[64] This is mainly due to relatively less concentration of oxygen at the site of enzymatic reaction required for the formation of hydrogen peroxide which can be explained from the consideration of exposed surface area of ormosil to solution and water contact angle. It has been reported that neither highly hydrophobic nor totally hydrophilic sol–gel matrices are desirable for sensing application.[41, 51] When chemical modifier such as metal dispersion, water-soluble polymers and proteins are added to the materials, the resulting electrodes become more hydrophilic and subsequently alter the water-contact angle which manifests the wettability. It has been reported[51] that a blank sol–gel electrode without any hydrophilic modifier shows the highest water contact angle (80°) and in turn lowest wettability (not amenable for nitrogen adsorption analysis) whereas the sol–gel electrode with all hydrophilic modifiers (carbon, poly(ethylene glycol), and Pd-GOD) shows the lowest water-contact angle (42°) and the highest wettability (42 m^2/g). An increase in wetted area increases the wetted conductive surface accessible to the solution and also the corresponding electrochemically active area and capacitive current. On the other hand, unwetted area does not contribute to the capacitive or faradic currents. The results reported in Fig. 2 follow similar arguments. Recently, Ingersoll and Bright have also reported the effect of dopant addition and used oxygen as analyte to study sensor performance.[52] The variation of oxygen concentrations has also been determined experimentally based on direct electrochemical reduction of oxygen in System-3 without glucose oxidase, in System-4 and at blank Pt surface. These results are shown in insets (II) and (III), respectively. Curves 1 and 2 of inset (II) to Fig. 2 show the oxygen reduction at System-3 without glucose oxidase and at the surface of System-4, respectively. It is clear that System-4 shows large cathodic peak current as compared to System-3 (curve 1) which shows relatively increased rate of oxygen diffusion within the wetted section of System-4. Finally, we also studied oxygen reduction at bare Pt electrode in same aqueous medium and the result recorded in inset (III) to Fig. 2 shows two electron transfer as reported earlier.[66, 67] These data demonstrate that the rate of oxygen diffusion increases from System-1 to System-4 in accordance to the data concluded from surface wettability area. The effect of water content on the sensing performance of ormosil-based glucose biosensor was also studied.[68] The increase in water content during ormosil processing enhances the performance of glucose sensing.

Actually, the configuration of the biosensors based on the present approach does not fulfil the requirement of mediated glucose biosensor; however, we have got some very interesting observations on the mediated bioelectrochemistry using soluble ferrocene and glucose oxidase immobilized within the ormosil of four different systems. We studied the electrochemistry of ferrocene monocarboxylic acid at the surface of ormosil-based glucose electrode (System-4).[64] Figure 3 shows the electrochemistry of soluble ferrocene monocarboxylic acid using System-4 respectively, at different scan rates (3, 6, 10, 20, 50 & 100 mV/s) between –0.2 and 0.6 V vs Ag/AgCl. Before the start of the measurements, each enzyme-immobilized sol–gel glass elec-

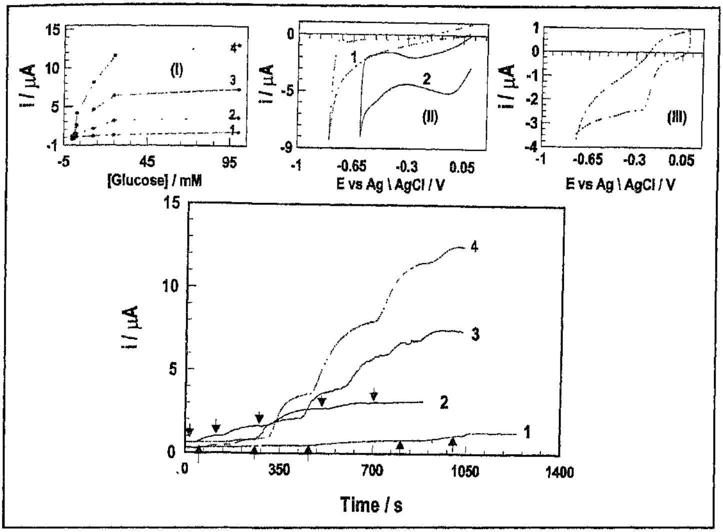


FIG. 2 Typical amperometric response of sol–gel glass-based glucose biosensors of four different compositions Systems-1, 2, 3 and 4. The recording shows typical response curve on the addition of concentrations of glucose. Inset (II) shows the cyclic voltammograms of sol–gel-modified electrodes showing oxygen reduction in 0.1-M phosphate buffer pH 7.0 at the scan rate of 5 mV/s; curve 1 was recorded from graphite powder-dispersed sol–gel-modified electrode without glucose oxidase, whereas curve 2 was recorded using System-4. Inset (III) is a cyclic voltammogram showing oxygen reduction recorded using bare graphite disk electrode (dia. 3 mm).

trode was allowed to incubate for 2 h in ferrocene solution to attain steady-state mass-transfer kinetics within the wetted area of sol–gel glass. The voltammograms of these systems were recorded in the same ferrocene solution. The inset to Fig. 3 shows the plot of anodic peak current vs square root of scan rates. The results shown in the inset follow the same trends as described by Wang and Pamidi.[56] Linear relation is obeyed between peak current and square root of scan rates although the linear line does not pass through the origin which shows that the systems are not very well diffusion controlled. The peak separation increases with scan rates, but decreases from System-1 to -4 from 84 mV to 60 mV, respectively, at the same scan rate. Wang and Pamidi[56] have also reported the peak separation of 57 to 65 mV in two sol–gel systems and linear lines obtained from the plots of peak current vs square root of scan rate also do not pass through the origin. The voltammograms show reversible electrochemistry of ferrocene in each system with diffusion-limited conditions in each case as evidenced from the result shown in inset. The data recorded in Fig. 3 show that peak current is relatively high in System-4 associated to relatively faster diffusion of ferrocene as compared to Systems-1 to -4 in accordance with the conclusion drawn based on non-mediated mode of electrochemical reaction

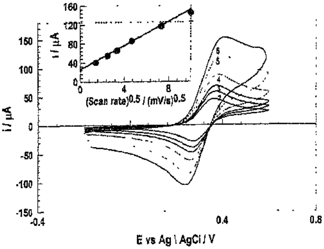


FIG. 3 Cyclic voltammograms of 5 mM ferrocene monocarboxylic acid in 0.1-M phosphate buffer pH 7 1 on sol–gel glass-modified electrode made from System-4 at 25°C at the scan rate of 3, 6, 10, 20, 50 and 100 mV/s. The inset shows the plot of anodic peak current vs square root of scan rate.

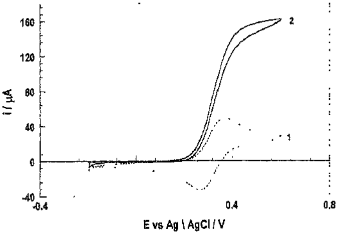


FIG 4. Cyclic voltammogram of GOD-immobilized composite sol–gel glass (System-4) in the absence (1) and presence (2) of 150 mM glucose in 0.1 M phosphate buffer pH 7.1 containing 5 mM ferrocene monocarboxylic acid at the scan rate of 5 mV/s

discussed above. Subsequently, we examined the relative mediated electrochemical response of the four systems in the presence and absence of 150 mM glucose. The cyclic voltammograms using System-4 in aqueous solution of ferrocene monocarboxylic acid between –0.2 and 0.6 V vs Ag/AgCl at the scan rate of 5 mV/s are shown in Fig. 4. Curve 1 of each voltammogram shows the recording in absence of glucose whereas curve 2 shows the recording in the presence of glucose. There is a large increase in anodic current on the addition of glucose in each system showing the mediated electron exchange from immobilized glucose oxidase. Here again[64] the variation in the magnitude of anodic peak current is much larger in System-4 (Fig. 4) again supporting our conclusion discussed above. In this case, the rate of glucose diffusion is relatively more rapid and apparently keeping second-order rate constant for the reaction between reduced glucose oxidase and oxidized ferrocene in all the four systems, the concentration of glucose in System-4 at the site of enzymatic reaction is greater than all other systems (1–3). Thus, the incorporation of polyethylene glycol and graphite powder significantly affects the morphology of the sol–gel glass-based biosensor that is related to the introduction of hydrophilic modifiers in order to control the thickness of the wetted section of the electrode (reaction layer thickness). Additionally, the incorporation of graphite particles not only increases the wetted surface area of the electrode, but also facilitates electron transfer within the sol–gel matrix as a result of increased electronic conductivity of the electrode. Finally, it is important to study the selectivity of the glucose oxidase encapsulated within the organically modified sol–gel glasses. The selectivity of the encapsulated GOD within sol–gel glass is deter-mined using sucrose, fructose, and *β*-D glucose. There is no response on the addition of 100 mM sucrose and fructose whereas good amperometric response is observed on the addition of glucose

7. Ferrocene-linked/encapsulated ormosil

The sol–gel materials used in electrochemistry have been reviewed.[69] A description of recent advances in various fields of sol–gel electrochemistry[54] and wiring of glucose oxidase to carbon matrices via sol–gel-derived redox-modified silicate have been reported. The encapsulation of redox material within sol–gel glass has been used in sensor design. Pankratov and Lev[70] reported tetrathiafulvalene-mediated CCEs with limited storage and in-use stability. Several other reports on the encapsulation of ferrocene within sol–gel glasses are available,[69–72] none dealing with reversible electrochemistry of ferrocene within sol–gel-based nano-matrix. Audebert *et al.*[71–72] reported modified electrodes from organic–inorganic hybrid gels containing ferrocene unit covalently bonded inside a silica network and modified electrodes from organic–organic hybrid gels formed by hydrolysis-polycondensation of some trimethoxysilylferrocenes.[72] Collinson *et al.*[73] reported electroactivity of redox probes encapsulated within sol–gel-derived silicate film based on anionic, i.e. [Fe(CN))$_6^{3-/4-}$]; [IrCl$_6^{2-/3-}$] and cationic, i.e. ferrocenemethanol [FcCH$_2$OH$^{0/+}$] gel-doped probes. Other reports on ferrocene-based sol–gel sensors are also available.[74] There is great potential to study ferrocene-encapsulated/linked sol–gel glasses for sensor applications.

We have reported a glucose biosensor based on bilayer of ormosil matrix[65] of controlled porosity within which an enzyme layer was sandwiched. We studied both mediated and non-mediated response of glucose biosensor.[64] The mediated response was based on the use of soluble ferrocene for the regeneration of reduced glucose oxidase. However, co-immobilization of mediator together with glucose oxidase does not permit the occurrence of mediated electrochemical reaction associated to restricted translational degree of motion within nanoporous geometry of sol–gel glass ormosil which is essentially required in the development of mediated enzyme biosensors. Accordingly, immobilization of ferrocene derivatives within sol–gel glass network with good reversible electrochemistry is an attractive requirement. We developed two systems using (1) ferrocene carboxaldehyde and a mixture of two silanes (3-aminopropyltrimethoxy silane and 2-(3, 4-epoxycyclohexyl)-ethyltrimethoxy silane and (2) ferrocene monocarboxylic acid and a mixture of 3-glycidoxypropyltrimethoxysilane, and trimethoxysilane. The electrochemistry of ferrocene based on cyclic voltammetry is reported.[75] These modified sol–gel glasses with and without ferrocene are characterized by scanning electron microscopy.[75, 76] These ferrocene-encapsulated ormosils were used for the development of

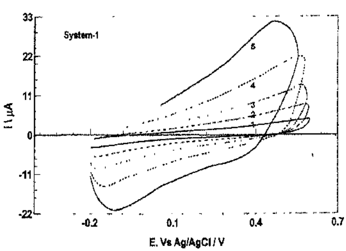


FIG. 5a. Cyclic voltammograms of ferrocene-linked sol–gel glass (System-1) in 0.1 M phosphate buffer pH 7.0 at 25°C at different scan rates (mV/s): (1) 20, (2) 50, (3) 100, (4) 200 and (5) 400.

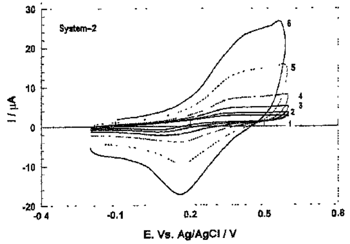


FIG. 5b. Cyclic voltammograms of ferrocene-encapsulated sol–gel glass (System-2) in 0.1 M phosphate buffer pH 7.0 at 25°C at different scan rates (mV/s): (1) 3, (2) 6, (3) 10, (4) 20, (5) 50 and (6) 100.

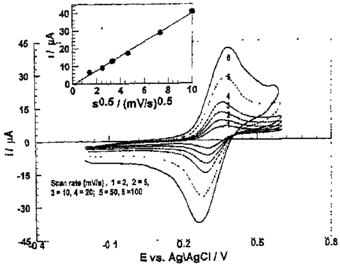


FIG 6. Cyclic voltammograms of ferrocene-encapsulated Pd-linked ormosil in 0.1 M phosphate buffer pH 7.0 at 25°C at different scan rates (mV/s): (1) 3, (2) 6, (3) 10, (4) 20, (5) 50 and (6) 100 The inset shows the plot of anodic peak current vs square root of scan rate.

ion sensor based on the approach described earlier.[77] The electrochemistry of ferrocene-linked ormosils are shown in Fig. 5. Figure 5b shows quasi-reversible electrochemistry of ferrocene as compared to ferrocene-linked ormosil (Fig. 5a) which shows capacitive cyclic voltammogram. Subsequently, we developed ferrocene-encapsulated palladium-linked ormosil, which was developed using Pd-linked sol–gel precursors[76]. The electrochemistry of the resulting ormosil is reversible with a peak separation of 58 mV (Fig. 6). The inset to Fig. 6 shows the plot of peak current vs square root of the scan rate. This ferrocene-encapsulated ormosil was used to develop electrocatalytic biosensor.[76, 78–79] The electrochemistry of encapsulated ferrocene within new ormosil shows production of novel material applicable to enzyme electrodes. A rigorous analysis of palladium linkage to 3-glycidoxypropyltrimethoxysilane is under way to provide deeper insight into exceptional structural and functional behavior of new material.

7. Conclusion

The review surveys the evolution of sol–gel glass chemistry which deals with novel materials of practical application. Ormosil and its application in sensor/biosensor design are reviewed. The availability of desired functional groups in ormosil precursor could be exploited in anchoring molecular-recognising receptor and for attaching sensing elements on optrode, electrode and on several other transduction surfaces especially for mimicking biological processes. Future projection on the production of redox moieties–metal–ormosil network especially using palladium, ruthenium and similar others is required for the development of sensors suitable for molecular recognition. Additionally, ormosil-based ceramic material analogous to conventional ceramic glasses could be derived at room temperature with the option of conducting nonceramic forming reactions for specific purposes during ormosil casting.

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