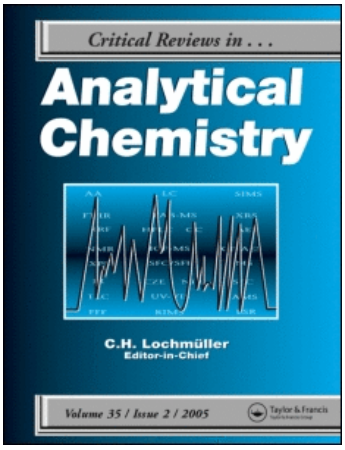

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Immobilization of Biomolecules in Sol–Gels: Biological and Analytical Applications

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The encapsulation or generation of new surfaces that can fix biomolecules firmly without altering their original conformations and activities is still challenging for the utilization of biochemical functions of active biomolecules. Presently, sol–gel chemistry offers new and interesting possibilities for the promising encapsulation of heat-sensitive and fragile biomolecules (enzyme, protein, antibody and whole cells of plant, animal and microbes); mainly, it is an inherent low temperature process and biocompatible. The typical sol–gel process initiates by the hydrolysis of $M(OR)_4$ and is performed in the presence of the active biomolecule. Hydrolysis and condensation of the M-monomers in the presence of an acid or base catalyst trigger cross-linking with formation of amorphous MO_2, a porous inorganic matrix that grows around the biomolecule in a three-dimensional manner. This class of sol–gel matrices possesses chemical inertness, physical rigidity, negligible swelling in aqueous solution, tunable porosity, high photochemical and thermal stability, and optical transparency. These attractive features have led to intense research in the optical and electrochemical biosensors, which may be useful for medical, environmental and industrial applications. On the other hand, sol–gel encapsulated organelles have been transplanted to the living systems, and plant/animal/microbial cells have also been employed for the production of commercially important metabolites. This review article highlights the advantages, recent developments, applications and future perspectives of sol–gel immobilized biomolecules, which includes enzymes, antibodies, microorganisms, plant and animal cells.

Keywords sol–gel, biosensors, antibodies, biosil, cells, entapment/encapsulation, enzymes, immobilization, immunosorbent, proteins

INTRODUCTION

Interdisciplinary knowledge is becoming more and more important to circumvent the newly emerging problems in medical, environmental and industrial sectors. Critical care is one of the most challenging (and stressful) areas of medicine, e.g., while giving the treatment to the patient, careful diagnosis of the marker such as protein and other disease-causing agent in the biological fluids is a deciding factor. Similarly for the early notice of warfare agents (chemical and biological agents), pollutants and toxins in the environments and isolation and development of new drugs in industrial sectors, the development of highly specific detection and quantification methods are challenging tasks for the analytical and bioanalytical scientists and academicians. To achieve this task i.e., development of new analytical methods/sensors needs—more than ever before—the extended interdisciplinary cooperation between the experts in the fields. Therefore, analytical and bioanalytical chemistry has been pronounced as an interdisciplinary and practice-oriented area. In recent years, much effort and research has been carried out on biosensors for the development of quick detection tools. Biosensors are analytical devices composed of a biological sensing element (enzyme, antibody, DNA) in intimate contact with a physical transducer (optical, mass or electrochemical), which together relates the concentration of an analyte to a measurable electrical signal (1–8). Almost similar kinds of biological recognition and catalytic properties, artificial receptors, have also been developed and used for different applications such as biosensors, chromatography, etc (9–11). Employing the biomolecules such as enzyme/antibody/microbe/plant/animal cell, as sensing element is highly beneficial because they had inherent properties such as specific recognition and catalytic activity (12). Another beneficial advantage with whole cells such as microbes/plant/animal cells is production of commercially valuable metabolites by growing them in controlled conditions (13, 14). During growth, sometimes cells lose their activity due to the physicochemical and mechanical (fermentor agitation/aeration) perturbations. Usually at the end of fermentation, cells contain still

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biological activity and can be used when they are in immobilized form (15).

In aqueous solutions, biomolecules such as enzymes lose their catalytic activity rather rapidly, because enzymes can suffer oxidation reactions or its tertiary structure could be destroyed at the air/water interface, hence making the use of enzyme and reagents both expensive and complex (16). These problems can be minimized considerably by enzyme immobilization. Similarly the other bioactive molecules such as antibodies, are also not much stable in aqueous solutions even though affinity is good towards the antigen and functionality. By attachment to an inert support material, bioactive molecules may be rendered insoluble, retaining catalytic activity, thereby extending their useful life (17–19). In view of the above necessity and advantages, since the 1960s, an extensive variety of techniques have been developed to immobilize biomolecules, including adsorption, covalent attachment and entrapment in various polymers (20–23). In general, adsorption techniques are easy to perform, but the bonding of the biomolecules is often weak and such biocatalysts lack the degree of stabilization and easy leakage from the matrix. Perhaps it is possible by entrapment or covalent attachment methods. But the covalent linkage method is tedious, often requires several chemical steps, and sometimes the involved compounds inactivate or reduce the activity of biomolecules. Direct immobilization of active biological substances such as enzyme, proteins, cells antibodies etc., in porous metal oxide carrier by physical entrapment via the sol–gel processes has drawn great interest in recent years. This is due to its simplicity of preparation, low-temperature encapsulation, easy for immobilization, chemical inertness, tunable porosity, optical transparency, mechanical stability, and negligible swelling behavior (24–29). The two major advantages with a sol–gel system is that it can retain a large content of water; this feature makes the encapsulated bio-recognition agents or enzyme catalytic centers long-term stable (24, 30) and the process can be performed at room temperature (25, 31). Other advantages of silica supports include biocompatibility and resistance to microbial attack. Moreover, the preparation conditions of a sol–gel have a remarkable effect on the activity of the entrapped active biomolecules. Hence several studies have been carried out to examine the properties of the porous sol–gel matrix, such as pore size distribution, surface area, pore geometry, morphology, and polarity (32–37). Using sol–gel techniques different nonrecognition agents such as enzyme (38–40), antibody (41, 42), and whole cells (43–45) have been successfully immobilized, employed for multiple applications, e.g., biosensors (46, 47), solid phase extraction sorbents (48, 49), production of commercially valuable enzymes and drugs (13, 50), etc.

In recent years several reviews on sol–gel technology have appeared with specific applied areas (24–26, 38, 51–56). It is extremely difficult to include all the aspects of sol–gel technology and cover every report; however, we have tried to incorporate the most important aspects. The present article highlights the advantages, recent developments, applications and future perspectives of sol–gel entrapped biomolecules, including enzymes, antibodies, microorganisms, plant and animal cells.

SOL–GEL PROCESS AND MATRIX CHARACTERISTICS

The immobilization of biomolecules in silicate glass formed by the sol–gel method has been employed in a number of works (24, 38). In general, the sol–gel process involves hydrolysis of alkoxide precursors under acidic or basic conditions, followed by condensation and polycondensation of the hydroxylated units, which leads to the formation of a porous gel. Typically a low-molecular weight metal alkoxide precursor molecule such as tetramethoxy silane or (TMOS) or tetra ethoxysilane (TEOS) is hydrolyzed first in the presence of water, acid catalyst and mutual solvent (24, 53). Hydrolysis of metal alkoxide (e.g., TEOS or TMOS) precursors results in the formation of silanol groups (Si-OH); through condensation, these silanol moieties react further and form siloxanes (—Si—O—Si—); finally through polycondensation of silanol and siloxanes, SiO_2 matrices are formed after aging and drying processes as shown in equations 1–3 and Figure 1. The resulted sol–gel is an interconnected rigid network with pores of sub-micrometer dimensions and polymeric chains whose average length is greater than a micrometer. HCl and ammonia are the most generally used catalysts for the hydrolysis; however, other catalysts such as acetic acid, KOH, amines, KF, and HF are also used (31). The rate and extent of the hydrolysis are mostly influenced by the strength and concentration of the acid or base catalyst (57). Usually weaker acids require longer reaction times to achieve the same extent of reaction compared to strong acids. Base-catalyzed hydrolysis of silicon alkoxides proceeds much more slowly than acid-catalyzed hydrolysis at equivalent catalyst concentration (32, 57).

$$(RO)_3SiOR + H_2O \rightarrow (RO)_3SiOH + ROH \quad [1]$$

$$2(RO)_3SiOH \rightarrow (RO)_3Si\text{-}O\text{-}Si(OR)_3 + H_2O \quad [2]$$

$$(RO)_3SiOH + ROSi(OR)_3 \rightarrow (RO)_3Si\text{-}O\text{-}Si(RO)_3 + ROH \quad [3]$$

When the liquid in the pore is removed at or near ambient pressure by thermal evaporation, drying and shrinkage occurs, the resulted monolith is termed as xerogel. If the liquid is primarily alcohol, the monolith is termed as an "alcogel." Usually, xerogels are superior in mechanical properties and chemical resistance to hydrogels in view of cross-linking and densification (24, 25). But the drying of hydrogels inevitably reduces porosity, increases steric compression and diffusional limitations, and results in a reduced bioactivity, especially for inorganic sol-gels (25, 26, 53). The animal/plant cells or organelles cannot survive at complete dehydration, and are very sensitive to structural disturbances caused by invasive polymer matrixes, and cells needed hydrophilic sol–gel/hydrogels to preserve their viability (38).

Silica sol–gels are transparent, chemically inert, negligible swelling in organic solvents compared to most organic polymers and mechanically stable. In most cases, they are appropriate

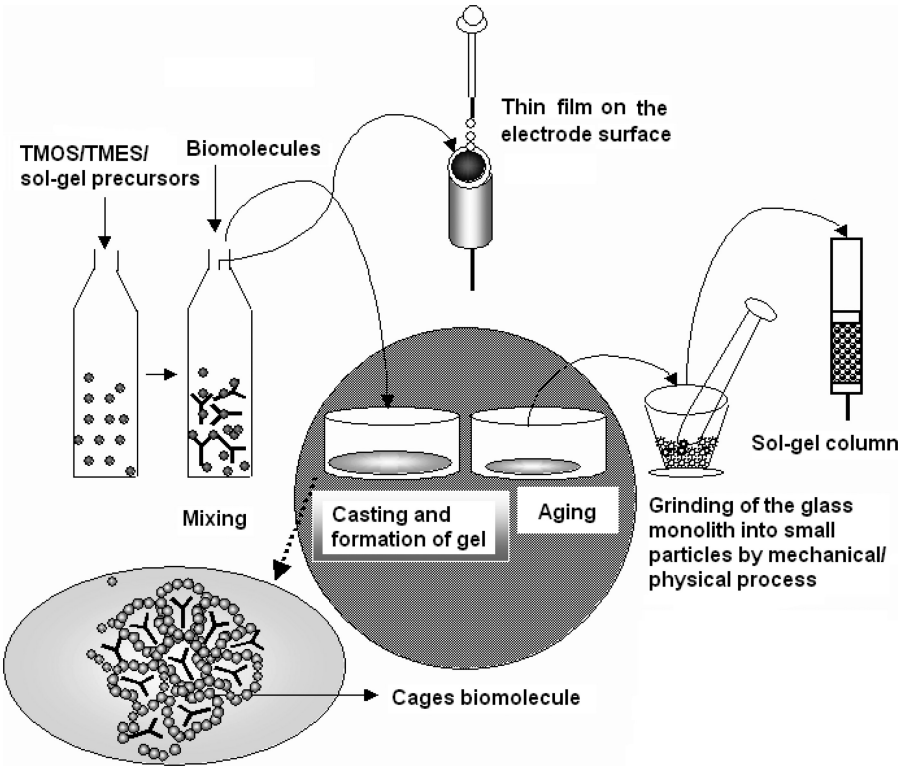

FIG. 1. Schematic diagram of the sol–gel process.

matrices for bio-encapsulation even though they are brittle in nature. In recent years, silica sol–gel-based inorganic-organic hybrid materials have also been reported (58). The sol–gel materials are generally based on silica, alumina, titania and other compounds. The introduction of various functional groups into organic alkoxide has led to organically modified sol–gel glasses (ormosils) (59). The use of ormosils in bioencapsulation may provide interesting properties to the host matrixes from hydrophobic to hydrophilic (hydrogels) (60, 61). 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 (62). On the other hand, when hydrophilic monomers are used, there is an increase in the water-wetted area of the sol–gel glass. Thus the ratio of hydrophilic and hydrophobic monomers in an organically modified sol–gel glass is crucial in the preparation of biocompatible matrices and sensor design (63). The structure and properties of doped sol-gels depend not only on the chemical compositions of the starting materials, but also on many operational factors involved in the preparations such as water/silica molar ratio, solvent, catalyst, pH and temperature. These parameters highly influence the hydrolysis and condensation and allow the control of nano- and microstructure of the final material (25). Such control is essential for achieving a proper balance between non-leaching of the entrapped bioactive molecules and its accessibility to the analyte. In addition to these the sol sitting time, gel-drying time, and the conditions under which the gel is aged and dried (i.e., relative humidity) can also affect the long-term performance of these materials (64).

Advances in the Sol–Gel Process

The major obstacle with sol–gel entrapment of biomolecules is the formation of alcohol as a by-product during the hydrolysis and condensation of the alkoxide precursors, which causes a detrimental effect on the activity of the biomolecules (54, 65). To circumvent this problem poly(glyceryl silicate) (PGS) sol–gel precursors have been introduced by Gill and Ballesteros (38). The stable, water-soluble PGS were prepared by the partial hydrolysis and condensation of tetramethyl orthosilicate (TMOS) to poly (methyl silicate) (PMS), followed by its transesterification with glycerol, in a one-pot reaction, catalyzed by hydrochloric acid or poly(antimony(III) ethylene glycoxide). PGS rapidly hydrolyzed and gelled in aqueous, buffered milieu without the need for any catalyst, to form silica hydrogels, which produced transparent, mesoporous, and physically stable silica xerogels after aging, washing to remove glycerol, and drying. These sol–gel materials showed good porosity, less shrinkage and high percentage of bioencapsulation, and the entrapped biomolecules retained almost complete activity (98%). The poly (silicic acid) entrapped thermolysin, lypoxigenase, silalic acid aldolase, tyrosinase and *S. salmonicolor* cells performed poor results due to the protein precipitation and premature/partial gelation. However, the PGS-derived silical matrices exhibited mild encapsulation chemistry and high precursor biocompatibility, and reduced

significantly the toxic effects. The biogels retained 83–98% of the activity of the native biological as compared with 11–76% for PMS (38). Later Liu and Chen reported alcohol free aqueous colloidal sol–gel process and encapsulated cytochrome c, catalase, myoglobin and hemoglobin with good retained activities (65). In another alcohol free sol–gel approach sodium silicate was employed as starting precursor (66), in which proteins showed preserved activity. Perhaps both PGS and sodium silicate, routes have inherent limitations in their applications, the glycerol-derivatized silicate precursors need to be synthesized, and in sodium silicate, route precursors release high Na^+ concentration, which must be eliminated through an acidic cation-exhange resin. In addition to this, none of these routes are suitable for the preparation of hybrid organic-inorganic matrixes that can provide comfortable environments for a number of biomolecules (67, 68). Ferrer et al. reported another alcohol-free and simple aqueous sol–gel method. In this approach the alcohol formed during the hydrolysis was removed through the rotavapor method (69). The HRP immobilized in this alcohol-free route exhibited a completely preserved activity and showed higher specific activity compared with regular sol–gel method. This method was applicable for the preparation of pure silica matrixes as well as ormosil. Overall, alcohol-free methods enabled the encapsulated proteins to retain their structure and biological activity for a prolonged period.

Some non-silica sol–gel materials have also been developed to immobilize bioactive moelcues for the construction of biosensors (70) and to synthesize new catalysts for the functional devices (71, 72). Liu et al. (70) proved that alumina sol–gel was a suitable matrix to improve the immobilization of tyrosinase for detection of trace phenols. Titania is another kind of non-silica material easily obtained from the sol–gel process (73). Yu and Ju (74) reported a simple and mild titania sol–gel thin film through vapor deposition in neutral medium. The titania sol–gel composite film is very efficient in retaining the activity of horseradish peroxidase (HRP) and preventing it from leaking out of the film. This method has been extended for the immobilization of hemoglobin (75), carcinoma antigen 125 (76) and carbohydrate antigen 19-9 (77) for the development of biosensors.

Luckarift et al. (78) reported a new enzyme immobilization (entrapment) method in a biomimetic silica support. The biosilicification method was very fast, mild and convenient for the entrapment of biomolecules at ambient temperatures. In this process, precipitation was catalyzed by the R5 peptide, the repeat unit of the silaffin, which was identified from the diatom *Cylindrotheca fusiformis*. During the enzyme immobilization in biosilicification, the reaction mixture consisted of silicic acid (hydrolyzed tetramethyl orthosilicate), R5 peptide, and enzyme. In the process of the precipitation reaction, the enzyme was entrapped and nm-sized biosilica-immobilized spheres were formed. This work used butyrylcholine esterase as a model enzyme. Compared to free enzymes, the biosilica-immobilized enzyme was stable up to 65°C for 1 hour, whereas free enzyme lost 85% of activity. These biosilica nano-spheres could be useful for the immobilization of other fragile and highly sensitive biomolecules and used for biosensor applications (78). Recently Prieto-Simon et al. (79) reported metal-modified 3-aminopropyltrimethoxysilanes (APTMS) that could be employed in the electrochemical monitoring of glucose and hydrogen peroxide. In this study copper-, iron-, zinc-, and cerium-modified APTMS were mixed with tetraethoxysilane (TEOS) and the finally produced xerogels. However, these gels had potential drawbacks such as lack of homogeneity, low adhesion onto the electrode surface, and cracking effects during aging. The incorporation of poly(ethylene glycol)-cellulose acetate composite solution (PEG/CA) improved the characters of these gels along with more wettability. The metal modified sol–gels could be more promising in development of interference-free biosensors. Carturan et al. (29, 43, 80) developed a biosil method for the encapsulation of plant and animal cells. The gas phase biosil process was recently applied to the bioencapsulation of animal cells in silica-alginate beads (81), and reviewd by Coradin et al. (49).

Advantages and Disadvantages with Different Sol–Gel Matrices

Different kinds of precursors and matrices have been developed to obtain the sol–gel matrices with improved properties like reduced brittle nature, high transparency, improved hydrophilicity, flexible porosity, etc. Different sol–gel matrices such as inorganic, organically modified (ormosils), hybrid sol–gels and interpenetrating polymer networks have been used for the encapuslation. Perhaps each type of sol–gel has its own advantages and disadvantages (26, 59). Inorganic sol–gels are good in transparency; chemical robustness but brittleness and low porosity in xerogels are major limitations. Similarly organically modified sol–gels have good tunable porosity and electrochemical activities, perhaps being relatively fragile and of limited optical transparency (27, 63). Hybrid sol–gels can be prepared with flexible rigidity, controlled porosity and balance hydrophobicity and hydrophilicity, but poor optical transparency and structural collapse on drying are somewhat limiting factors. Interpenetrating polymer networks are combined matrices of sol–gel with water-soluble polymers such as carrageenan, alginate, agar, PVA and PEG, etc. These matrices are highly biocompatible for fragile molecules such as organelles and living cells. Compared with alginate and carrageenan beads, sol–gel-layered beads are stable against chelating agents and physicochemical perturbations due to the supporting action of outer sol–gel layer (29, 82). For the improvement of conductivity and mechanical strength, sol–gels are generally filled with nano- or micro-particulates platinum, palladium, graphite and methylated silica, clays and cellulose etc., respectively (reinforced matrices). Plant cells encapsulated in polyester fibers reinforced sol–gel films can be employed in the production of enzymes in which reinforced polyester fibers serve as a support for the sol–gel films and anchorage for the plant cells (82).

Porosity of Sol–Gel

For the better performance of encapsulated biomolecules, the doped silica-matrix pores need to meet two requirements. Pores should be large enough to allow unrestricted transport of molecules including buffer ions, substrates and products of the reaction and analytes; simultaneously, it should exclude large particles such as bacteria. Second, pores should be small enough to prevent leakage of encapsulated macromolecules (24, 25). Generally most of the entrapped enzymes show an increase in K_m, which means high substrate concentration compared to native enzyme (66). Behind this a number of factors are involved, including partitioning of substrates between solution and support, and diffusion resistance to the transport of substrates to the enzyme. The presence of small pores, or bottlenecks even in big pores, can reduce the diffusion coefficients of substrates and products significantly in a silica matrix. If the dimensions of the matrix are large or if there are a large number of very small pores, enzyme molecules buried inside the matrix encounter a substrate concentration significantly lower than that at the surface. If the diffusion rate of the substrate is sufficiently slow compared to enzymatic catalysis, the enzyme molecules close to the surface can use up most of the substrate molecules entering the matrix, effectively making the substrate concentration be zero in the interior of the matrix (66). The similar transport or diffusional problems arise in case of antigen/antibody reaction, plant and animal cell bio-transformation or production of primary or secondary metabolites, when entrapping the respective biomolecules in sol–gel matrixes. To overcome these problems, pore size and density should be controlled for their better performance. Different agents including surfactants and non-surfactants have been used as pore-improving agents. Various alcohols and mixed solvent systems have also been used for the improvement of pore size (33). Along with these, many synthetic routes and strategies have been developed to yield a wide diversity of materials of various framework chemical compositions and pore structures. In most of the studies, ionic and neutral surfactants have been employed as templates (83, 84), which direct the mesophase formation based on the electrostatic and hydrogen-bonding interactions, respectively.

Jie et al. (85) developed macropore-sized (100 μm) sol–gel bioglasses using poly(vinylalcohol) (PVA) as a pore-forming agent. Changing PVA/sol ratio could further control pore size. Takahashi et al. (86) reported that the incorporation of PVA into lithium niobate ($LiNbO_3$) can obtain thick films along with crack-free, smooth and good diffusional properties. Xi et al. (32) studied the effect of different catalysts such as HCl, NH_4OH, HNO_3, HF and NH_4F on TEOS sol–gel pore size. Recently Soares et al. (87) entrapped *Candida rugosa* lipase in TEOS and MTMS (methyltrimethoxysilane) in presence of PEG. It showed considerable esterification activity due to the increased mean pore size and improved protein accessibility. Wei et al. (88) doped a non-surfactant D-glucose as a pore-forming agent into the sol–gel. The doping of glucose was highly beneficial as it provided a biocompatible environment, could be easily removed under mild conditions and could be used to develop low-cost materials. Tetraalkyl orthosilicate in the presence of non-surfactant template (glucose) could improve the activity of entrapped alkaline phosphatase (ALP) more significantly than in conventional microporous sol–gel materials (89).

On the other hand, during the aging process, cross-linking of the network increases and the internal solvent is expelled from the matrix, causing the internal polarity and viscosity to be changed and the average pore size to be decreased in a manner that depends on the aging conditions (90, 91). The nonuniformity of the pores in silica sol–gel matrices causes cracks and fractures in dry monolithic sensors upon immersion in water (25, 62). To solve this problem, some researchers used surface-active drying control chemical additives such as Triton-X and quaternary ammonium compounds or copolymers to prepare the sol–gel film (92, 93). Previous studies revealed that in usual sol–gel processes, the pore sizes of xeorgels and hydrogels (wet/aged gels) were between 2–20 nm and 4–100 nm (26). Proteins such as cytochrome *c* and RNAse (94), and antibodies (95, 96), could reversibly immerse into the sol–gel and could selectively bind with the doped moelecules. Rao and Dave (97) reported that proteins could selectively bind with the sol–gel matrix and diffuse in and out. Bis[3-(trimethoxysilyl)-propyl]ethylenediamine (enTMOS) derived sol–gel exhibited the selective affinity behavior of protein with globular heme proteins (cytochrome *c*, myoglobin and hemoglobin). Collagen membranes coated with SiO_2 layers showed diffusion of proteins, and diffusion coefficient reduced with increasing protein size (molecular weight). The substantial reduction was obtained at MW 150,000. This kind of material is beneficial in medical applications for designing artificial organs (98).

Interactions Between Sol–Gel Matrix and Analytes

The interactions between sol–gel matrices and analytes include electrostatic, hydrogen bonding and hydrophobic interactions. They play key roles in the accessibility of analytes to the entrapped biomolecules even though the pore sizes are quite enough for the analytes to pass though the matrices, and are important in determining the accessibility of analytes to entrapped proteins. In cases where repulsive or attractive interactions exist between the glass and analytes, the sol–gel matrices take up the analytes either partially or excessively (25, 28). The study of these interactions is very important because they affect the diffusional properties significantly. Badjic and Kostic (99, 100) studied the interactions between polar silica and organic compounds. Silica monoliths immersed in solutions containing styrene are evenly dispersed, as styrene cannot form hydrogen bonding with silica. After soaking of silica matrix in electrolyte solutions at a pH value at which pore walls are negatively charged, anions such as $[Fe(CN)_6]^{3-}$ are only partially taken up, whereas cations such as $[Ru(NH_3)_6]^{3+}$ are excessively taken-up by the sol–gel matrix from the surrounding solution. In either case internal and external concentrations of the ion are unequal even after equilibrium is reached (101). The protein entrapped in TEOS

gels completely showed accessibility to the neutral quenchers, perhaps partial accessibility or repulsion in case of negatively charged quenchers (102).

Stability of Biomolecules in Sol–Gel

Physical entrapment of proteins in a sol–gel matrix preserves protein structure and functionality and protects the protein from physicochemical perturbations. It is mainly due to the sol–gel matrix "cages," which provide more rugged environment to the dopant. The sol–gel entrapped heme proteins such as cytochome *c* (cyt *c*) and myoglobin (Mb) showed good stability against pH and thermal perturbations compared to protein in solution (65, 103). Usually globular proteins such as cyt *c* contain noncovalent interactions that maintain the native folded state under physiological conditions. The disruption of these interactions by heating or chemical treatment leads to the conformational change and eventually denaturation. But the sol–gel caged cyt *c* shows high thermal stability due to the exact fitting of the protein inside the cage, which is controlled by the protein size (104). As the heme proteins retain their reactivity in optically transparent glass, sol–gel encapsulated Mb can be employed as a sensing element for the measurement of dissolved oxygen in water using optical spectroscopy (105).

ALP entrapped in SiO_2 xerogels shows improved thermal stability at basic conditions. The half-life at 70°C and pH 9.0 is 2.6 minutes for free enzyme, 4.7 minutes for the entrapped state. The entrapped ALP retains activity for 2 months at optimal conditions. In another study sol–gel encapsulated acid phosphatase also exhibits pronounced protective effect. Enzyme trapped in sol–gel glasses (in presence of PEG 400 and NaF) is stable upto 12 minutes at 70°C in 0.1 M pH 5.6 citrate buffer, whereas it is stable only for 0.1 min in solution (106, 107). Bovine carbonic anhydrase II (BCA II) is not unfolded even at 74°C, retains 33% activity; perhaps it was unfolded in solution at 64°C. The encapsulated BCA II obeys the Michaelis–Menten kinetics by hydrolyzing the *p*-nitrophenyl acetate (108). This enhanced stability is due to the protective nature of the cage and the rigidity of the SiO_2 matrix, which reduces the freedom of peptide-chain refolding molecular motions (24). Trypsin and acid phosphatase entrppled in silicate sol–gel along with PEG have half-lives 100-fold times higher than that of enzyme in solution at 70°C (106). More interestingly the creatine kinase (CK) encapsulated in TMOS sol–gel exhibits 4-fold improved activity upon short exposure to the elevated temperatures. Circular dichroism results indicate the initial conformations of CK in sol–gel and solution are different, and the entrapped enzyme activity is not at maximum. However, after heat-treated at 47°C the CK encapsulated in sol–gel matrix reaches maximum activity after 10 hours. It is mainly due to the improvement in the pore size, the rearrangement of the CK conformation during this process and the interactions between enzyme and sol–gel matrix (109). Through resonance Raman spectra Das et al. (110) proved that myoglobin could be preserved in native form even at lower pH by encapsulating them in sol–gel glasses. Chen et al. (111) studied in-depth the stability of three flavoprotien oxidases, i.e., glucose oxidase (GOD), lactate oxidase, and gluconate oxidase in hydrated silica gels. The half-life of the GOD at 63°C increased up to 200-fold after immobilization. But the lactate oxidase and gluconate oxidase activities were improved only after the doping of weak base poly (*N*-vinylimidazole) and strong base poly (ethyleneimine), respectively, prior to the immobilization. The protein stabilization depended mainly on the charges present on the protein and active sites and their interactions with anionic silica matrix. GOD is zwitterionic in nature, hence it is more stable in caged gels. Different types of additives have been employed as stabilizers to the entrapped proteins, including ligand-based stabilizers (Cod III parvalbumin (112), oncomodulin (113), methyltrimethoxysilane-based materials (to stabilize atrazine chlorohydrolase) (114), the incorporation of organosilanes and polymers into lipase-doped silica (115), poly-(ethylene glycol) (to stabilize acetylcholinesterase and butyrylcholinesterase) (116) and graft copolymers of polyvinylimidazole and polyvinylpyridine (to stabilize entrapped glucose oxidase and horseradish peroxidase) (93, 117). Recently Brennan et al. (118) reported the addition of sugar (sorbitol) and amino acids (*N*-methylglycine) increased the thermal stability and improved the α-chymostriosin and RNAse T1 activity, because the added osmolytes (sorbitol, *N*-methylglycine) altered the hydration effects, protein silica interactions and pore morphology.

The sol–gel entrapment method is biocompatible and shows improved thermal stability to the bioactive molecules, however partial inactivation or loss of activity is a quite common problem. Ferrer et al. (119) observed the activity loss of HRP immobilized in TEOS gel films and reasoned that the reduction in activity was due to the detaching of a part of heme groups from active sites during the initial sol–gel formation steps. When these heme groups (HRP-encapsulated aged gel) were exposed to working buffer, they leached out within a day; later, no further reduction in activity was observed even after extensive washing, which was monitored though heme characteristic absorption at 280 nm, and tryptophan fluorescence studies. In the cases of some enzymes, active and inactive protein aggregation also causes loss of enzyme activity. Lloyd and Eyring (120) controlled intermolecular protein-protein interactions by immobilizing HRP in a silicate matrix. Usually the sol–gel pores were large enough to allow diffusion of reactants to the enzyme but small enough to prevent the enzyme leakage and interact/aggregate with other enzyme molecules.

Dynamics of Proteins in Sol–Gel

The entrapped protein vicinity is completely different from the native environment. Hence, the potential use and development of robust analytical devices, the conformational, rotational and transalational dynamics and the accessibility of the entrapped proteins should be closely monitored. The conformational and dynamic motions of the entrapped proteins have been examined widely using absorbance, fluorescence

(35), resonance Raman (110), dipolar relaxation (37), and time-resolved fluorescence anisotropy (35) measurements. After encapsulation, proteins such as bovine serum albumin (BSA), human serum albumin (HSA) and monellin retain their conformation (112, 113, 121, 122), but small molecules such as myoglobin (Mb) (121) undergo substantial conformational changes during the entrapment. On the other hand, small proteins such as cytochrome *c* and parvalbumin appear to be able to retain full conformational flexibility upon entrapment and are only moderately affected by aging of the matrix (37, 112). It may be due to the collective effect of high alcohol concentration, aging and pore size. The sol–gel cage restricts the conformational change of big molecules, which leads to partial unfolding, but small molecules can easily be gotten more conformational changes/denaturation (28). Edmiston et al. (121) studied the behavior of myoglobin and acrylodon-labeled bovine serum albumin (BSA-Ac) entrapped in TMOS derived xerogels. These composites were subjected into the solutions that contain ionic quenchers or chemical denaturants. Then the protein response and conformation were detected by following the static emission spectra and intensity (121). Jordan et al. (28) observed the nanosecond and picosecond dynamics of BSA-Ac and acrylodan-labeled human serum albumin (HSA-Ac) when they were sequestered within sol–gel-derived xerogel glasses. These experiments indicated that the "global" protein rotational motion was not arrested within a xerogel. In another study Baker et al. (123) reported that the protein mobility could be modulated by using polymer-doped xerogels. The dynamics of HSA entrapped in TEOS-derived materials has recently studied by Flora et al. (102). The entrapped protein showed no global motion (hindered rotation), and performed only segmental and local rotational motions in the region of Trp 214. They reasoned that it was attributed to electrostatic or other interactions between the protein and the silica, which restricted global rotational motions of the protein. It was also suggested that the protein likely underwent some unfolding upon entrapment, leading to a greater degree of segmental motion in the protein. Brennan and coworkers (124) have recently shown the real-time behavior of monellin sequestered within thin TEOS-derived xerogels as the protein is challenged by the quencher acrylamide and the chemical denaturant guanidine hydrochloride. The rotational mobility of GOD and its active site FAD have been investigated by Hartnett et al. (125) GOD caged in sol–gel, rotational mobility is reduced 2-fold times more than in solution, perhaps the active site pocket is similar to that in solution. Santangelo et al. (126) studied the active site dynamics of horse heart ferricytochrome *c* encapsulated in silica hydrogels. They reported that the dynamics were highly dependent on the structure of water trapped in the hydrogel. The controlled PEG added in TMOS-derived sol–gel showed improved dynamics of the pyrene, rhodamine 6G (R6G) and acrylodan-labelled bovine serum albumin (BSA-Ac) (123). The time-resolved fluorescence results of R6G and BSA-Ac within TMOS composites showed that relative to free, TMOS R6GB was more mobile in PEG-doped composites. With the increasing of the PEG doping, the R6G and BSA-Ac also exhibited faster dynamics. In this study PEG did not affect the pore diameter but was well tuned and the sol–gel processed composite dipolarity and altered the mobility of dopants. These results were further interpreted as being due to the preferential adsorption of the PEG to the silica surface, which resulted in enhanced mobility for the entrapped protein, due to less protein–silica interactions (123). Hence while optimizing the influencing parameter for encapsulated biomolecules, dynamics of the proteins inside the "cage" should also be taken into consideration for their better performance.

SOL–GEL IMMOBILIZED BIOACTIVE MOLECULES

Enzymes

Since emerging in sol–gel bioencapsulation, enzymes have been widely studied due to their well-known biochemical mechanisms, commercial availability, wide applications, easy solubility in water, good stability, simple molecules compared with animal, plant or microorganisms. In 1955 Dickey (127) demonstrated for the first time bioencapsulation with partial activity of urease and catalase, but muscle adenylic acid deaminase completely lost its activity. After 3 decades, considerable attention has been paid again towards the bioencapsulation using sol–gel glasses. Avnir's group (128) successfully encapsulated alkanie phosphatase in silica gel, which retained its activity up to 2 months (30% of initial) with improved thermal stability. Further Shtelzer et al. (129) sequestered trypsin within a binary sol–gel-derived composite using TEOS and PEG. In the same year Zink et al. (130) entrapped other proteins cyt *c* and Mb in TEOS sol–gel. Later studies on bioencapsulation were accelerated by other groups, and several proteins such as Mb (25, 104, 105), Hb (25, 104, 131, 132), cyt *c* (103, 133), lactate oxidase (134), GOD (79, 125), ALP (135), Cu-Zn superoxide dimutase (25), urease (136), bacteriorhodopsin (bR) (137, 138), HRP (139, 140) and acetylcholinesterase (141), etc. were immobilized into sol–gel matrices. Previous reports described the various aspects of sol–gel-entrapped biomolecules in such properties as conformation (122, 142), dynamics (35, 143), accessibility (121), reaction kinetics (101, 124), activity (24, 144–146), and stability (114, 136, 147–149).

Biosensor Applications of Enzymes Entrapped in Sol–Gels. Thus far, sol–gel encapsulated enzymes have been widely employed in the construction of biosensors using different detection methods such as electrochemical and optical methods (24, 25, 28, 38). The matrix inherent features such as optical transparency, high surface area, chemical and photochemical inertness and the ability to obtain any desired shape and form (monoliths, thin films, powders, fibers), enabling the design optical sensors (62, 150–153). The slow diffusion of the electroactive species inside the sol–gel matrix causes a long response time. Hence for the improvement of conductivity, metal particles such as graphite, gold, palladium, iridium, etc. have been doped into sol–gel matrices in the construction of electrochemical

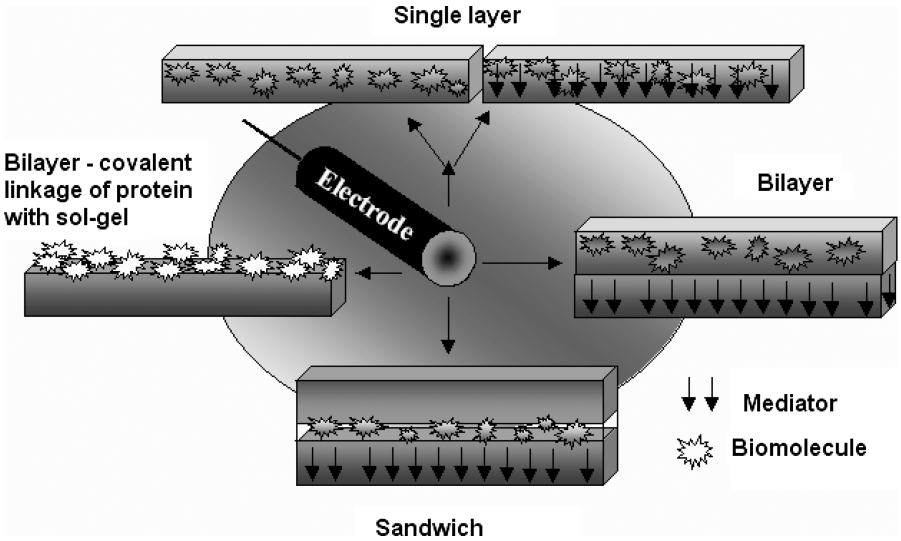

FIG. 2. Different configurations employed for the coating of sol–gel films on the electrode surface.

biosensors (154–157). Gavalas et al. (158) doped carbon nanotubes as conductive materials in to alkoxy silane sol–gels. L-amino oxidase was encapsulated into CNT-sol–gel through and an aqueous sol–gel process. The added particles improved morphology of the composite surface and electrochemical characteristics. Sol–gel-derived electrochemical biosensors mainly rely on two basic configurations: conductive ceramic composites (156, 159, 160), and electrode surface coatings (161, 162). Since the pioneering work of Lev and coworkers sol–gel-derived composite carbon electrodes (CCEs) (163–165) have been widely used to develop all kinds of amperometric biosensors. For fixing the entrapped enzyme on the electrode surface, three main approaches dependent on the convenience, stability and response are followed; they are single layer (166, 167), bilayer, (154) and sandwich (147, 168) configurations (Figure 2). Along with entrapment inside the sol–gels, few of the studies have been carried out by covalently linking the active biomolecules on the surface of the sol–gel glasses (154). Another important factor in developing biosensors is the thickness of gel film. In most cases, thin films exhibit quicker response than the thick layers due to the good diffusion properties (42, 169, 170). With increasing gel thickness, the signal decays and diffusion of analytes to an biomolecule active site becomes difficult; eventually these factors lead to poor response. Homogeneous distribution of enzymes is possible in sol–gel films compared with regular carbon paste electrodes; hence, it allows the homogeneous electron transfer and larger current response in amperometric sensing.

Among all the enzymes studied, GOD and HRP have been widely used in sol–gel entrapment studies. The sol–gel immobilized GOD exhibits high thermal stability in terms of half-life (200-fold longer than that in the free state) mainly due to the favorable local polymer–protein interaction between the positive charges on the GOD and the negatively charged silicate matrix (171). Brun et al. (150) reported an optical biosensor based on the xerogel disk doped with GOD, peroxidase and dye for the detection of glucose. Tatsu (172) prepared tetraethyl orthosilicate derived silica gel doped with GOD and used it as a glucose recognition element in flow-injection analytical system. Metal alkoxides are more attractive matrices because they provide good conductivity and the possibility of manipulation of polarity, rigidity, pore size and distribution and electronic conductivity (173). Using the advantages of metal alkoxides, Glezer and Lev (174) prepared platinum electrodes coated with vanadium pentoxide sol–gel film doped with GOD. Liu et al. (170) reported a glucose biosensor based on immobilization of enzyme in alumina (aluminium iso-propoxide) sol–gel films on a platinized glassy carbon electrode. At the GOD/sol–gel/platinized GCE, glucose response started at +0.2 V approached a maximum value at +0.6 V. The low operating potential greatly minimized the interference from coexisting electroactive species. Liu et al. (176) immobilized HRP in the ZrO_2 sol–gel matrix to develop a biosensor for the H_2O_2 determination. Ju and coworkers (74, 177) introduced a simple vapor-depsoition method to immobilise HRP, GOD and other biomolecules titania sol–gel, which retained their catalytic activity and exhibited good response on electrode surface (Figure 3). The uniform porous structure of the titania sol–gel matrix had very low mass transport barrier, a high catalytic activity, and a fast response (74). Narang et al. (147) developed a glucose biosensor by immobilizing GOD between two sol–gel layers (sandwich). By talking advantage of ormosils, Pandy et al. (178) reported a glucose biosensor based on the immobilization of GOD in a sol–gel glasses derived from 3-aminopropyltrimethoxy silane and 2-(3,4-epoxycyclohexyl)-ethyltrimethoxy silane. These films had unrestricted diffusional properties, and smooth surface face without cracking.

The 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

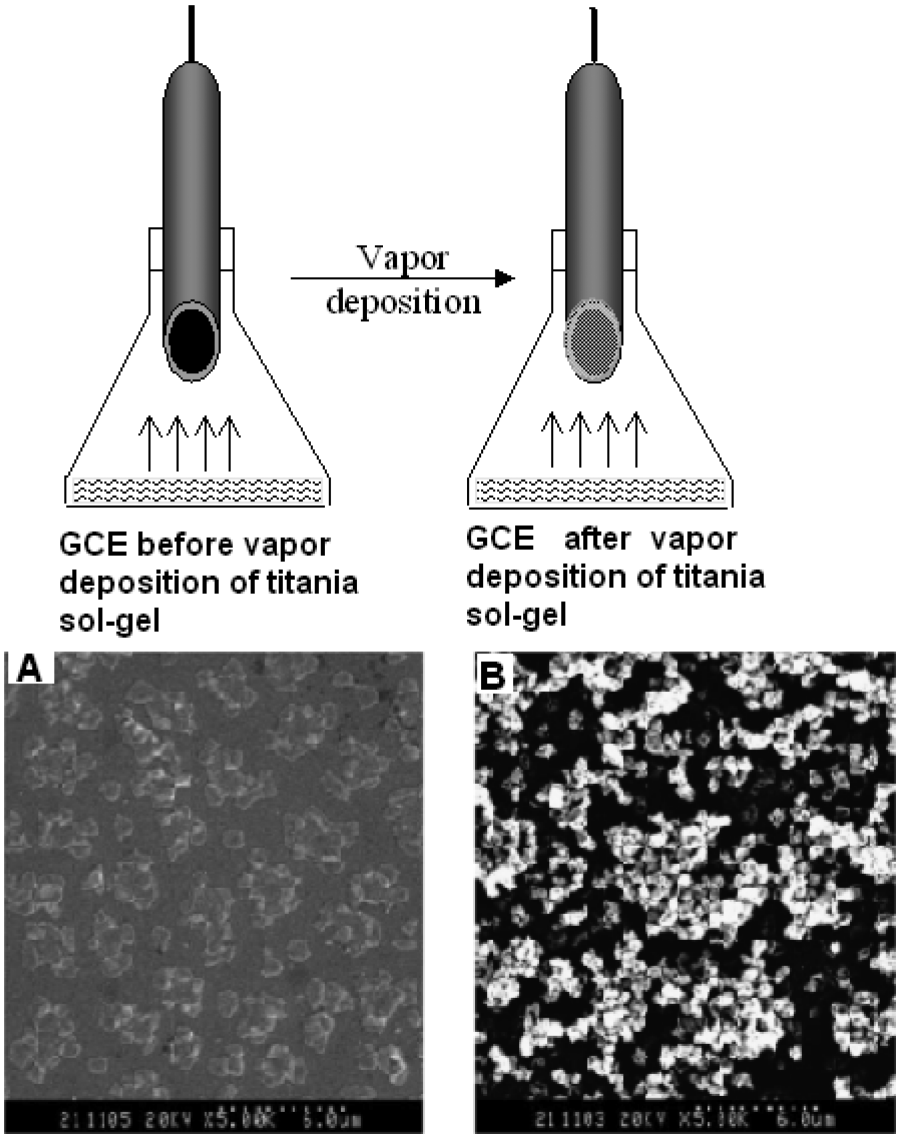

FIG. 3. Schematic digram of glucose oxidase immobilized in Titania sol–gel prepared with vapor depostion method on glassy carbon electrode. (A) Titania sol–gel film and (B) GOD in Titania sol–gel.

mediated biosensor (179, 180). Mediators such as ferrocene and its derivatives, osmium complexes, quinone salts and dyes, and ruthenium examine have been employed as bisensors (163, 181–185). Sensors in which the mediator is coimmobilized with enzyme on electrode will not require the addition of the former to the test solution, thereby eliminating many practical limitations. The immobilized mediator catalyzes the redox reactions of biomolecules and shuttles the electrons more efficiently between enzyme and electrode to greatly enhance the sensing performance. On the other hand, greatly the inclusion of electrocatalytically active graphite-supported palladium, platinum, and ruthenium or gold nanocrystals allowed the construction of mediator-free electrodes that are highly sensitive, selective, and stable (156, 163, 186). Mediatorless amperometric biosensors have been reported by coupling GOD with HRP or soybean peroxidases, which mediate the reduction of hydrogen peroxide (163, 168, 187). The fundamental problem arising in the construction of an amperometric glucose biosensor is the selectivity of the substrate detection. The often-used detection approach is electrochemical oxidation of the liberated hydrogen peroxide through the enzymatic reaction, and requires a relative high working potential. At such a potential, endogeneous or exogeneous compounds present in biological samples (for example, urate, ascorbate or paracetamol) can be electrochemically oxidized, leading to a high level of interference and false results in the quantification of glucose concentration. To overcome this limitation, Wang et al. (188) and Lev (189) took advantage of the catalytic properties of palladium-modified carbon

particles to detect glucose at lower potentials (+0.3, +0.5 V) via a screen-printing process or by molding the porous organically modified silica in a glass capillary. Coche-Guerente et al. (187) reported a mediatorless amperometric bienzymatic (GOD and HRP) glucose biosensor, in which graphite particles were doped as electrical communicators between the electrode surface and HRP active center. HRP and GOD were layered separately on electrode surface in two layers, one on another. Ju et al. (190) have also reported a mediatorless phenol biosensor based on titania sol–gel encapsulated tyrosinase. Recently the direct electrochemistry of sol–gel encapsulated proteins such as Hb has been reported. The immobilized Hb displays excellent electrocatalytic (peroxidase) performance to the reduction of O_2, NO_2^- and H_2O_2 (191, 192). By taking advantage of the direct electrochemistry of HRP, Ju et al. have developed reagentless immunosensors for the detection of carcinoma antigen 125 (76), and HCG (193). These kinds of reagentless immunosensors are highly useful for medical applications, because the use of fewer reagents in clinical assays leads to less interference and more accuracy, especially in real samples such as serum, urine and other biological fluids. Another possible way to develop reagentless biosensors is to immobilize sensitive fluorophore into the silica sol–gel matrix along with bioactive molecules. The changes in the enzyme reaction mixtures affect the fluorophore response. The co-immobilization of dextran conjugates of fluorescein or carboxy-seminaphtharhodafluor-1 (SNARF-1), and enzyme showed less leaching and quantifiable pH response of fluorescence in reaction mixtures of lipase and urease (194). Another pH sensitive fluorescent biosensor was reported for the detection of acetylcholine and praoxon (195), in which fluorescene isothiocyanate (FITC)-Dextran conjugate and acethylcholinesterase (AChE) were coimmobilized in TMOS sol–gel. This biosensor showed a linear range from 0.5 to 20 mM for achetylcholine and 30% inhibition of AChE acitivty at 152 ppb of paraoxon.

To circumvent cracking and swelling, Lev et al. (196) reported some composite ceramic-carbon materials along with surfactants, but these materials were less biocompatible, and the surfactants were detrimental to the enzyme and needed high amounts of enzyme (92, 163, 197–199). Hydrogels show promising results in retaining the enzyme activity; perhaps the swelling nature limits the practial biosensor application (200–204). Recent reports address that the hybrid silica sol–gels are favorable for the retaining the activity of biomolecules and fast and sensitive response of the biosensors (93, 94). To improve the hydrophilic nature and to reduce the cracking and swelling, grafting copolymer poly(vinyl alcohol) grafting 4-vinylpyridine has been incorporated into the sol–gel (TEOS) and entrapped GOD (93). This polymer retains well the activity of entrapped enzyme and is able to firmly adhere to the electrode surface (93, 205). The biosensor based on GOD entrapped in the titanium oxide and poly(vinyl alcohol) grafting 4-vinylpyridine (PVA-g-PVP) composite matrix shows quick response (<20 s) and a linear range for glucose detection up to 9 mM with a sensitivity of 405 nA/mM. PVA-g-PVP grafting polymer provides hydrophilic-hydrophobic properties and large amount of hydrogen bonds inside the sol–gel, hence the enzyme retains its active configuration (206). Kurokawa et al. (207) reported an improved stability and lifetime of GOD on electrode surface by the addition of cellulose into titanium oxide composite, but the main drawback of this biosensor was longer time (>0.5 h) required to reach a steady state because the fiber was rigid and dense, which limited its application in analysis of practical samples on-line. Very recently the entrapment of GOD in hydroxyethyl carboxymethyl cellulose (HECMC) and TEOS hybrid polymer drastically improved the stability of GOD (up to 3 years). The aging of the sol–gel matrix at 4°C resulted in reduction of shrinkage and good porosity. When integrating this encapsulated enzyme with optical biosensor, it showed good linearity for glucose quantification between 50 to 200 μM in urine (208). In hybrid sol–gel matrices, organic components contribute to the formation of defect-free inorganic membranes and make it less brittle, and an inorganic phase can improve the chemical and temperature stability of organic membranes (209). Similarly, to obtain improved properties, pure silica sol–gels have been doped with natural polymers such as chitosan. Miao and Tan (210) incorporated chitosan into TMOS-derived sol–gels. The biocompatible features of chitosan helped in retaining the HRP activity on carbon composite electrodes. In another study chitosan was crosslinked with (3-aryloxypropyl) dimethoxymethylsilane, in which HRP was sequestered for the construction of H_2O_2 biosensor (211). Chen et al. (212) fabricated a glucose biosensor by encapsulating the GOD in MTMOS and natural polymer chitosan compsosite matrix and using ferrocene as a mediator. All the cases of biomolecules encapsulated in sol–gel hybrid materials showed improved stability and good analytical response due to the improved characteristics of matrix.

For the long-term use of enzyme biosensors, one of the hurdles is fouling and contamination of the surface during operation. The two advantageous alternatives to dissolve this are polishable/renewable biosensors and disposable biosensors. Polishable biosensors can be renewed by mechanical removal of the outer surface; renewable amperometric biosensors are commonly comprised of either carbon paste or carbon-epoxy materials. Sampath and Lev reported (213) a renewable GOD-entrapped glucose biosensor by the addition of hydrophilic PEG into the hydrophobic ormosil (present on the electrode surface). Li et al. (214) reported MTMOS-derived a carbon composite-based renewable glucose biosensor using vinyl ferrocene as a mediator. Low-cost, screen-printed materials are often used as disposable biosensors (215). Wang et al. (188) reported, for the first time, a screen-printed electrode modified with GOD/HRP that was stable up to 3 months. The renewable or screen-printed sensors were highly convenient for the filed applications and low cost. In order to circumvent limitations such as microbial adhesion and growth on the surface, recently nitric oxide (NO)-releasing sol–gel particles has been reported (216). Nitric oxide is a potential antimicrobial agent (217), if it releases slowly from

the matrix adhesion and the growth of microbes can be controlled. NO-releasing glucose biosensors prepared by doping diazeniumdiolate-modified sol–gel particles in a polyurethane membrane exhibits high sensitivity (mention details), reproducibility and fast response up to 18 days. To reduce both enzyme inactivation by NO (by minimizing NO exposure) and sol–gel particle leaching, polyurethane membranes are coated as a layer (217).

Photoactive Proteins. Photoactive proteins such as bacteriorhodopsin (bR) and phycoerythrin (PE) have had their mechanisms studied extensively, for potential use as active components of photonic devies. PE and bR retain their optical activity when encapsulated within sol–gel glasses, with enhanced stability against phtodegradation (218). Bacteriorhodopsin is a naturally occurring transmembrane protein that converts light energy into metabolic energy. It was found in the photosynthetic system of a salt-marsh bacterium called *Halobacterium salinarium*. In its native form, the bR molecule is located in a cell membrane commonly called "the purple membrane." Within the bacterial cell, bR is critical to the survival of the organism in an oxygen-deficient environment, as the bR molecules function as light-driven proton pumps, which transport protons across the cell membrane. This generates a proton, which in turn produces an electrochemical potential used by the organism to synthesize adenosine triphosphate (ATP). Effectively, bR is used by the bacterium to directly convert sunlight into chemical energy. The absorption of light initiates a photocycle in the bR molecule, which accompanies the transportation of protons. The characteristics and effects of this photocycle make it a potentially useful material for development as an optically sensitive film that is self-developing and erasable. The encapsulation of light-sensitive proteins in transparent matrices is of interest because of the potential application to photovoltaic devices, photoimaging, molecular computing (219–221), and chemical sensing (222). Wu et al. (223) and Weetall's group (224, 225) entrapped bR in wet sol–gel glasses. The D96N mutant bR retained its activity in a dried sol–gel glass (226). Shamansky et al. (227) studied the D96N mutant bR kinetics in dried xerogels. Encapsulation of photochromatic proteins in transparent films can be employed in optoelectronic sol–gel devices, which seems to be technically feasible (228). Phycobiliproteins are biomolecular assemblies located on the outer thylakoid membranes of marine algae. The optical properties of these proteins can be used as fluorescent markers in biochemical and biomedical reseach. To interface optical properties of PE, Chen et al. (218) entrapped it in sol–gel materials. Although PE was stable under ambient light, it denatured at intense light illumination. The sol–gel entrapped PE retained its conformation, exhibited improved photodegradation capacity and was more stable than in solution. Such a stablilized protoactive proteins could be used in potential applications in biomolecular sensing, imaging and information processing and storage.

Use of Sol–Gel Entrapped Enzymes in Drug Screening. Another potential application of sol–gel-entrapped enzymes is drug screening. Besanger et al. (229) entrapped clinically important enzymes cyclooxygenase-2, Factor Xa, dihydrofolate reductase, and Á-glutamyl transpeptidase in TEOS and diglycerylsilane (DGS). DGS-entrapped enzymes showed high catalytic activity and stability. Perhaps K_m values increased due to the slower diffusion and portioning of the substrates within the microporous materials. However the interaction between inhibitor and entrapped enzymes was similar to the free enzyme in solution. Similarly in another study (230) for the first time Src protein tyrosine kinase was entrapped in DGS sol–gel. Entrapped Src protein tyrosine kinase activity was improved through ligand stabilization effect by the addition of ATP before encapsulation. The IC_{50} value of Src protein tyrosine kinase entrapped and free enzyme in solution were close (230). These studies indicates that the active sites of sol–gel encapsulated enzymes are accessible to the inhibitor and substrate in sol–gel "cages." Hence, such entrapped enzymes can be employed for the high-throughput drug screening by paying more attention towards entrapment of other enzymes. It cannot be generalized that all enzymes can behave similarly in sol–gel matrices such as Src protein tyrosine kinase, hence sol–gel parameters should be optimized according to the interactions of inhibitor with enzyme and sol–gel matrix.

Likely several enzymes have been encapsulated in different sol–gel matrixes for the different applications. In consideration of space limitations, other reports are presented in Table 1. Previous articles reported by Jin and Brennan (28) and Gill (26) well documented the biosensor applications through the year 2000. To avoid repetition in this article, we list biosensor applications of the sol–gel-encapsulated enzymes and other proteins from 1999 to date, to the best of our knowledge.

Bioconversion Using Sol–Gel Entrapped Enzymes. In the chemical industry a variety of catalysts for well-defined chemical transformations are required. These reactions do not or only do unspecifically occur under normal conditions, so that the desired product is available only in a small concentration or as a mixture with by-products. This often results in the need of costly purification processes, which makes the chemical synthesis economically non-efficient. An alternative possibility is the use of enzymes. These biocatalysts enable highly specific transformations under moderate reaction conditions. Because of the high stereo- or regio-selectivity, the application of enzymes may be superior to chemical synthesis. Sometimes enzymatic processes can be used for the production of compounds, which are difficult to synthesize chemically. Especially enzymatic methods are suitable for the synthesis of optically active compounds. Often only one enantiomer of a compound has a pharmacological effect, whereas the other enantiomere has no, or an unwelcome, effect. Therefore, biotechnical syntheses gain importance in the production of compounds requiring high enantiomeric purity for pharmaceuticals or commercially important products. A milestone in the area of enzymes as catalysts for organic chemistry is the discovery that some of them (e.g., Lipase) retain their catalytic activity in non-aqueous media (296, 297). Normally, lipases catalyze the hydrolysis of carboxylic acid esters with

TABLE 1
List of enzymes immobilized in sol–gel matrices for biosensor applications in recent five years

No	Biomolecule	Analyte	Sol–gel precursors and additional compounds used	Mode of detection	Linear Range (LR) and Detection Limit (DL)	Ref.
1	GOD	Glucose	TMOS/Prussian Blue/Sandwich	Amperometric	LR: 0–4-75 mM; DL: 0.02 mM	231
2	GOD	Glucose	TEOS/hydroxyethyl carboxymethyl cellulose polymer	Fluorescence/Optical	LR: 9.0 μM–100 mM; DL: 9 μM	232
3	GOD	Glucose	Ormosil-Trimethoxysilane, Glycidoxypropyltrimethoxysilane, ferrocenemonocarboxylic acid, graphite powder, Palladiumchloride	Amperometric	LR: 0–35 mM; Sensitivity: 56.7 μA cm^{-2} mM^{-1}	233
4	GoD	Glucose	Titanium isopropoxide/poly(vinyl alcohol) grafting 4-vinylpyridine (PVA-g-PVP)-tetrathiafulvalene-mediator	Amperometric	LR: 0–9 mM; Sensitivity: 405 nA/mM	206
5	GOD	Glucose	MTMOS/Polyurethane/Diazeniumdiolate-modified sol–gel particles	Amperometric	LR: 0–60 mM	216
6	GOD	Glucose	(3-mercaptopropyl) trimethoxysilane (MPS)/Copper nitrate/graphite powder	Amperometric	4.0×10^{-5} to 5.6×10^{-3} M; DL: 1.8×10^{-5} M	234
7	GOD	Glucose	Methyltrimethoxysilane (MTMOS), 3-aminopropyltrimethoxysilane (APTMOS) and ferrocene/covalent linkage	Cyclic voltammetry	LR: 0.1–27 mM; DL: 26 μM	235
8	GOD	Glucose	3-aminopropyltriethoxy silane (3-APTMS)/2-(3,4-epoxycyclohexyl)-ethyltrimethoxy silane (EETMS)/ polyethylene glycol	Amperometric/ flowinjection analysis	LR: 1×10^{-4} to 5×10^{-3} M	236
9	GOD & HRP	Glucose	TEOS	Fiber optical/ Chemiluminescence	LR: 0.2–2 mM; DL: 0.12 mM	237
10	GOD & HRP	Glucose	Methyltrimethoxysilane (MTMOS)/pyrrole/ferrocene carbosylic acid/graphite	Amperometric	LR: 8.0×10^{-5} - 1.3×10^{-3} M	238
11	GOD/Catalase	Glucose	TEOS	Thermometry	LR: 0.2–30 mM; DL: 0.06 mM	239
12	GOD	Glucose	Gamma-aminopropylmethyldimethoxysilane (APMDMOS) and tetramethoxysilicane (TMOS)/mediator toluidine blue O/glutaraldehyde	Amperometric	LR: 1 to 8 mM; DL: 5×10^{-2} mM	240
13	GOD	Glucose	TEOS	Chemicluminescence/ Optical	LR: 5.0×10^{-4} to 1.0×10^{-7} M ; DL: 4.0×10^{-8} M	241
14	GOD	Glucose	Zn-APTMS/TEOS-PEG/Cellulose acetate	Amperometric	LR: 4×10^{-6} to 1×10^{-3} M; DL: 2.0 μM	79
15	GOD	Glucose	Titanium isopropoxide/Nafion/ferrocenium mediator/vapor deposition	Cyclic voltametry	LR: 0.07 to 15 mM; Sensitivity: 7.2 mA cm^{-2} mM^{-1}	242

16	GOD	Glucose	Tetraethyl orthosilicate (TEOS)/luminol	Chemiluminescence/ Optical fiber	LR: 50 μM–10 mM; DL: 26 μM	243
17	GOD	Glucose	Methyl trimethoxysilane (MTMOS)/ferrocene	Amperometric	LR: 3.2×10^{-4} to 3.5×10^{-2} M; DL: 0.3 mM	183
18	GOD	Glucose	TMOS/GOD labelled fluorescein-5(6)-carboxamido-caproic acid N-hydroxisu-ccinimide ester	Fluorescence/Optical	LR: 100 to 1000 mg L^{-1}	244
19	GOD	Glucose	MTMOS/graphite/luminol	Chemiluminescence	LR: 0.01 to 10 mM; DL: 8.16 mM	245
20	GOD	Glucose	TEOS	Chemiluminescence	LR: 3.5–70 μM; DL: 0.6 μM	246
21	GOD	Glucose	Al_2O_3	Amperometric	LR: 4.00×10^{-5}–2.19×10^{-2} M; DL: 1×10^{-5} M	175
22	GOD	Glucose	MTMOS/Carbon powder/vinylferrocene	Amperometric	LR: 1×10^{-4} to 9.6×10^{-3} M; DL: 4×10^{-5} M	247
23	GOD	Glucose	TEOS/Nafion	Amperometric	UPLR: 500 mg dl^{-1} Sensitivity: 0.54 ± 0.14 nA mg^{-1} dl^{-1}	248
24	GOD	Glucose	Al_2O_3/BSA/polyurethane	Amperometric	UPLR: 40 mM; DL: 5.2 mM	249
25	HRP	H_2O_2	ZrO_2/alcohothermal route	Amperometric	LR: 2.5×10^{-7} to 1.5×10^{-4} M DL: 1.0×10^{-7} M	176
26	HRP	H_2O_2	(3-Mercaptopropyl)trimethoxy-silane/hydrazine	Amperometric	LR: 5×10^{-6} to 4×10^{-3} M; DL: 2×10^{-6} M	250
27	HRP	H_2O_2	TEOS/poly(vinyl alcohol) and 4-vinylpyridine/tetrathiafulvalene/thin film	Amperometric	LR: 0–1.3 mM; DL: 2.5×10^{-7} M	251
28	HRP	H_2O_2	Methyltrimethoxysilane (MTMOS)/ Polypyrrole/ferrocenecarboxylic acid	Ampperometric	LR: 9.0×10^{-7} to 2×10^{-4} M; DL: 5.0×10^{-5} M	252
29	HRP	H_2O_2	MTEOS/Osmium redox polymer-mediator	Amperometric	LR: 0.01–2.7 mM; DL: 0.005 mM	253
30	HRP	H_2O_2	TEOS/PVA-g-PVP/potassium hexacyanoferrate(II) mediator	Amperometric	LR: up to 3.4 mM; DL: 5×10^{-7} M	254
31	HRP	H_2O_2	TEOS/luminol	Chemiluminescence	DL: 6.7×10^{-4} M; LR: 0.1–3.0 mM	255
32	HRP	H_2O_2	TEOS	Chemiluminescence	LR: 0.01–2 mM; DL: 8 μM	256
33	HRP	H_2O_2	(3-mercaptopropyl)-trimethoxysilane (MPS)	Amperometric	LR: 5.0 μM to 10.0 mM; DL: 2.0 μM	257
34	HRP	Cyanide	Metyltiethoxysilane (MTEOS)	Amperometric	LR: 0.004–0.04 mM; DL: 0.5 μM	258
35	HRP	H_2O_2	(3-aoryloxypropyl) dimethoxymethylsilane/chitosan	Amperometric	LR: 5.0×10^{-9}–1.0×10^{-7} M; DL: 2×10^{-9} M.	259
36	HRP/Catalase	H_2O_2	Al_2O_3/methylene blue	Amperometric	LR: 0.01 mM–0.1 mM	260

(Continued on next page)

TABLE 1
List of enzymes immobilized in sol-gel matrices for biosensor applications in recent five years (Continued)

No	Biomolecule	Analyte	Sol-gel precursors and additional compounds used	Mode of detection	Linear Range (LR) and Detection Limit (DL)	Ref.
37	HRP	Phenolics	TMOS/luminol	Chemiluminescence	p-iodophenol, LR: 2.67–9.33 μM DL: 0.83 μM p-coumaric acid, LR: 3.3–266 nM; DL: 15 nM 2-naphthol. LR: 40-200 nM; DL: 48 nM	261
38	HRP	H_2O_2	TEOS/Nafion/methylene green	CV/Chronoamperometry	LR: 0.5 μM to 1.6 mM; DL: 1.0×10^{-7} M	262
39	HRP	H_2O_2	MTMOS/3-mercaptopropyltrimethoxysilane (MPTMS)/meldola's blue	Amperometric	LR: up to: 0.6 mM; DL: 9×10^{-7} M	263
40	Tyrosinase	Phenolics	TMOS/CTAB/mediators-ferrocene/cobalt(II)/ phthalocyanine/opper(II)/phthalocyanine/	Amperometric	Catechol, LR: 2×10^{-7}-2.6×10^{-5} M; Sensitivity: 1.45 A M^{-1} Phenol, LR: 4×10^{-7} to 2×10^{-5}; Sensitivity: 1.28 A M^{-1} p-cresol, LR: 4×10^{-7} to 1.5×10^{-5}; Sensitivity: 1.03 A M^{-1} m-cresol, LR: 4×10^{-7} to 1.5×10^{-5}; Sensitivity: 0.64 A M^{-1}	264
41	Tyrosinase	Phenolics	TiO_2 [Titanium tetrabutoxide ($Ti(OBu)_4$)]	Amperometric	DL: 1×10^{-8} M	265
42	Tyrosinase	Phenolics	TiO2/poly(vinyl alcohol) with 4-vinylpyridine	Amperometric	Catechol, upper limit of LR: 14 mM; DL: 0.5 μM p-Cresol, UPLR: 2.0×10^{-5} M; DL: 0.2 μM Phenol, UPLR: 14 mM; DL: 0.9 μM	266
43	Tyrosinase	Phenolics	Al_2O_3	Amperometric	LR: 5×10^{-9} to 3.5×10^{-4} M; DL: 0.2 nM	70
44	Tyrosinase	Phenolics	3-mercaptopropionic acid (MPA)/Glutaraldehyde	Amperometric	Catechol, LR: (2.0–1000) $\times 10^{-7}$ M; DL: 0.11 μM p-Cresol, LR: (6.0–800) $\times 10^{-7}$ M; DL: 0.23 μM 4-Chloro-3-methylphenol, LR: 0.2–20 μM; DL: 94 nM 4-Chlorophenol, LR: 0.4–40 μM; DL: 0.15 μM Phenol, LR: 0.2–200 μM; DL: 8 nM	267
45	Tyrosinase	Phenolics	Aluminium isopropoxide [Al_2O_3 sol–gel-$Fe(CN)_6^{4-}$]	Amperometric	Phenol: LR: 0.1 to 250 μM; DL: 5.0×10^{-8} M	268

46	Tyrosinase	Phenolics	TMOS/Nafion	Amperometric	Catechol, LR: 1–100 μM; DL: 0.35 μM Phenol, LR:5–100 μM; DL: 1.0 μM *p*-Cresol, LR: 1–50 μM; DL: 0.34 μM 4-Chlorophenol, LR: 5–50 μM; DL: 0.67 μM 4-Acetamidophenol, LR: 5–50 μM; DL: 5.0 μM	269
47	Tyrosinase	Phenolics	MTMOS/TEOS/PVA-g-PVP	Amperometric	Catechol, LR: 1×10^{-7} to 1×10^{-4} M; DL: 0.04 μM *p*-Cresol, LR: 1×10^{-7} to 2.3×10^{-4} M; DL: 0.05 μM Phenol, LR: 2×10^{-7} to 1.6×10^{-4} M; DL: 0.1 μM	270
48	Urease	Urea	TMOS/Screenprinted Interdigitated array	Conductometric	LR: 0.03–2.5 mM; DL: 30 μM	271
49	Urease	Urea	TMOS/IDA/gold electrode	Conductometric	LR: 0.2–50 mM	272
50	Urease	Cd(II) and Cu(II)	TMOS/FITC-dextran	Fluorescence/Optical	LR: 10–230 μM; DL: 10 μM	273
51	Uricase-HRP	Uric acid	TEOS/amplex red	Fluorescence	LR: up to 1 μM; DL: 20 nM	274
52	Uricase/HRP	Uric acid	TEOS/luminol	Chemiluminescence	LR: 1 to 100 mg L^{-1}; DL: 0.1 mg L^{-1}	275
53	Lactate dehydrogenase	Lactate	TEOS/sandwich	Thermal	LR: 0.5 to 3 mM	276
54	Lactate dehydrogenase	Lactate	TEOS/Polyaniline/Indium-tin-oxide (ITO)	Amperometric	LR: 1 to 4 mM	277
55	Lactate dehydrogenase	L-lactate	TMOS	Fluorescence	LR: 0.2 to 1.0 mM	278
56	Lactate oxidase	Lactate	TMOS/poly(ethyleneimine)	Amperometric	LR: 0.5–2.0 mM; DL: 0.03 mM	279
57	Monoamine oxidase	Benzydamine	3-aminopropyltriethoxy silane, 2-(3,4-epoxycyclohexyl) ethyl-trimethoxy silane, graphite powder/polyethylene glycol	Amperometric	At platinum, LR: 0.05–2.5 mM, At carbon paste electrode, LR: 0.1–2.5 mM	280
58	Alkaline phosphatase	Pesticdes	TMOS/inhibition studies	Fluorescence	Metham-sodium, LR: 194–774 μM DL: 4.9 μM Tetradifo, LR: 305-28 μM DL: 292.3 μM	281
59	Cholesterol oxidase	Cholesterol	TEOS/Prussian blue	Cyclic voltammetry	LR: 1×10^{-6} to 8×10^{-5}M; DL: 1.2×10^{-7} M	282
60	Cholesterol oxidase/HRP	Cholesterol	TEOS	Amperometric	LR: 2–10 mM; DL: 0.5 mM	283

(Continued on next page)

TABLE 1
List of enzymes immobilized in sol-gel matrices for biosensor applications in recent five years (Continued)

No	Biomolecule	Analyte	Sol-gel precursors and additional compounds used	Mode of detection	Linear Range (LR) and Detection Limit (DL)	Ref.
61	Soybean peroxidase	H_2O_2	TEOS/poly(vinyl alcohol) and 4-vinylpyridine/metylene blue mediator	Amperometric	LR: 0.02 to 2.6 mM; DL: 5×10^{-7} M	284
62	Superoxide dismutase	Superoxide anion	Na_2SiO_3/PVA	Amperometric	LR: 0.2–1.6 μM; DL: 0.1 μM	285
63	Creatininase and creatinase	Creatinine	Ormosil: 3-Aminopropyltrimthoxy silane, 2-(3,4-epoxycyclohexyl)-ethyltrimethoxy silane, polyethyleneglycol	Cycic voltametry	DL: 100 μM	286
64	Aldehyde dehydrogenase	Acetaldehyde	MTMOS/Meldola's blue	Amperometric/Screen printed	LR: 10–260 μM	287
65	Cholinesterase	carbaryl and dichlorvos	TEOS/phenyltrimethoxysilane (PTMOS)/glutaraldehyde/bromcresol purple/three-layer sandwich	Optic Fiber	Carbaryl, LR: 0.11–8.0 mg L^{-1}; DL 108 μg L^{-1} Dichlorvos, LR: 5.0–30 μg L^{-1}; DL: 5.2 μg L^{-1}	288
66	L-Amino acid oxidase	L-phenylala-nine	Propyltrimethoxysilane (PTMOS)/sodium silicate/carbon nanotubes	Cyclic voltametry	LR: 0.02–10 mM; DL: 0.02 mM	289
67	Xanthine oxidase	Hypoxanthine	TMOS/graphite	Amperometric	LR: 1×10^{-6} to 1×10^{-3} M; DL: 3.8×10^{-7} M	290
68	Hemoglobin	H_2O_2	TEOS/luminol	Chemiluminescence/ Optical	LR: 6×10^{-5} to 4×10^{-7}M; DL: 5×10^{-6} M	291
69	Hemoglobin	H_2O_2	Titanium isopropoxide/vapor deposition	Amperometric	LR: 5.0×10^{-7} to 5.4×10^{-5}M ; DL: 1.2×10^{-7} M	75
70	Hemoglobin	H_2O_2	TEOS/glycerol	Cyclic voltametry	LR: 1×10^{-6} to 2.8×10^{-4} M Sensitivity: 64.8 μA mM^{-1}	292
71	Hemoglobin	H_2O_2	TEOS/luminol	Chemiluminesce-nce	LR: 6×10^{-5} to 4×10^{-7} M; DL: 1.3×10^{-7} M	293
72	Glutathione *S*-transferase	Atrazine	TEOS/PTMOS/bromcresol green-indicator/glutaraldehyde/sandwich configuration	Fiber-Optic	LR: 2.52–125 μM; DL: 0.84 μM	294
73	*Trichosporon cutaneum* and *Bacillus subtilis*	BOD	TEOS/poly (vinyl alcohol) and 4-vinylpyridine (PVA-g-P(4-VP), biochemical oxygen demand	Amperometric	LR: 1.0–60.0 mg L^{-1}; DL: 0.5 mg L^{-1}	295

formation of carboxylic acids and alcohols. Lipases are most frequently used enzymes in organic chemistry, because they can make chemo-, regio- and stereoselective esterification or transesterification and also they make possible the catalysis of the enantioselective acylation of amines (298, 299). Reetz et al. (300) successfully immobilized different lipases in hydrophobic sol–gel matrices, with improved stability and pronounced degrees of enhanced activity in esterification and transesterification reactions in organic solvents. The improved activities were due mainly to the enhanced accessibility of enzyme due to the fine distribution inside matrices, and the interactions between hydrophobic groups present in sol–gel matrices and lipase active sites (115, 300). The lipase entrapped in TMOS (hydrophilic SiO_2 gel) retained only 5% activity; however, the incorporation of hydrophobic alkyl groups in the silicon dioxide matrix led to improved activity. When hydrophobic interfaces are absent, lipases have some elements of secondary structure ("lids") covering their active sites, making them inaccessible to substrates (301). In the presence of hydrophobic interfaces, conformational changes take place, yielding the "open structure" of lipases. In another study the lipase entrapped in hydrophobic sol–gel materials was employed in ester hydrolysis in aqueous medium. However during recycle, a reduction in ($\sim$ 5%) activity was reported, due to the combined effects of mechanical and loss and leaching (302). Usually after each cycle in transformation reactions, the catalyst has to be separated from the reaction mixture, for which the usual method is filtration. To simplify the separation, magnetite nanoparticles were entrapped along with lipase in methyltrimethoxysilane (MTMS) sol–gel matrices. Lipases were able to tolerate the presence of magnetite, thus heterogeneous biocatalysts could be easily separated magnetically from the reaction mixture (299).

Phyllosilicate sol–gel entrapped lipase could be used as a biocatalyst in conversion of recycled restaurant grease into alkylesters. The addition of molecular sieves improved yield due to the removal of water (303–305). Co-immobilization of enzymes is a convenient approach for the multi-enzyme processes. By co-immobilizing formate dehydrogenase, formaldehyde dehydrogenase and alcohol dehydrogenase in silica gels, together with the electron donor NADH, synthesis of methanol can be performed at low temperatures and pressures (306, 307). Kuncova et al. (301) studied the effect of different organic solvents on stericacid esterification using immobilized lipase. Solvents acetone and toluene impaired the activity of free lipase, whereas sol–gel immobilized enzyme partially lost the activity. The thermal stability of lipase increased 55-fold upon entrapment in propyltrimethoxysilane (PTMS)/TMOS sol–gel material. The half-life of immobilized lipase was 4 months at 40°C in hexane with efficient esterification capacity. This stability was not only due to the physical entrapment but also to additional multiple interactions with SiO_2 through hydrogen, ionic, or hydrophobic interaction, and through multi-point attachment to the support (308). Invertase can split sucrose into glucose and fructose. These two products are simple compounds and act as substrates for the production of aminoacids, organic acids, antibiotics, etc. Nakane et al. (309) entrapped invertase in the three-dimensional network of cellulose acetate (CA) and ZrO_2 fibers. The ZrO_2 fiber was formed by coordinated interaction between the zirconium and the oxygen of the hydroxyl group or the carbonyl group on CA molecules. The immobilized invertase retained inside the fibers with 92% of initial activity, even after 10 recycles and repeated washes. With the increasing of the protein concentration the activity of invertase was improved due to the homogeneous dispersion and completely restricted swelling. Recently Pierre (55) reviewed the sol–gel encapsulated enzymes and their applications. Even though the sol–gel entrapped enzymes show improved stabilities against physicochemical changes around their vicinity and activity; still, they are facing diffusion limitations, which lead to the high K_m value and have great impact on conversion at large scale use of immobilized enzymes in industrial applications. Hence more efforts are needed for the improved diffusion properties of sol–gel matrices for efficient biotransformation/synthetic reactions.

Antibodies

Antibodies (Ab) are immune system-related proteins called immunoglobulins. Each antibody consists of four polypeptides with two heavy chains and two light chains joined to form a "Y" shaped molecule. The amino acid sequence in the tips of the "Y" varies greatly among different antibodies. This variable region, composed of 110–130 amino acids, gives the antibody its specificity for binding antigen. Because of their high specificity and sensitivity they have been widely employed in number of modern diagnostic and therapeutic technologies such as in vitro diagnostics, like radioimmunoassay (RIA), immunoradiometric assay (IRMA), enzyme-linked immunosorbent assay (ELISA), or bloting techniques. In addition to this antibodies have been employed in affinity chromatography (310, 311), cell typing and graft rejection identification (312), detection of different cells and disease markers (313), drug delivery and disease control (314), immunosensors (315, 316) etc. Usually antibodies are produced through immunization in vertebrates or hybridoma technology (monoclonal antibodies). In the last 10 years, progress in understanding the cellular and molecular basis of the immune response has made it theoretically possible to generate antibodies against almost any compound. Recombinant DNA technology allows the antibody binding properties to be modified at the DNA level in suitable organisms such as *E.coli* or yeast cells making it possible to obtain an antibody rapidly with desired properties without need for the immunization of the animals.

Immobilization of Abs on to a solid support was first reported in 1967 (317), and the technology has widespread application in affinity chromatography (AC) and other areas. However the major problem associated with covalent immobilization of antibody on solid surface is partial loss of biological activity due to the random orientation of the asymmetric macromolecules, sometimes steric hindrance caused by the neighboring antibody molecules

(318). Compared with covalent attachment of the antibodies, entrapment is quite mild and can reduce steric hindrances. In view of the inertness and biocompatibility of the sol–gel entrapment technique antibodies have been successfully encapsulated in the sol–gel materials for different applications. The Abs encapsulated in sol–gel-derived glasses can interact with target molecules with a high degree of specificity as in solution, and the signal can be detect using an appropriate sensing scheme (42). Generally antibodies are high molecular weight proteins, if antigen is also high molecular weight compound the interactions between antibody and antigen is difficult through the small pores of the matrix. Sometimes even though antigen is small compound, the tagged proteins (enzyme) have high molecular weight, which hinders the antigen-antibody interaction. In such a case, the antigen can be tagged with small signaling compounds such as FITC, ferrocene or their derivatives etc, depending upon the detection mode. Wang et al. (319) encapsulated firstly antifluorescein antibodies in TMOS sol–gel. The entrapped antibodies retained activity significantly and could bind with fluorescein molecules, which led to the decrease in the fluorescence.

To evaluate the diffusion of the fluorescein and the decrease in fluorescence due to the antigen-antibody complex or adsorption on to the silica marix, sol–gel was prepared with and without antibody, and the affinity was checked. The gels dried in desiccator lost 90% fluorescence due to the conformational changes of the sol–gel entrapped antifluorescein (Ab), and the antifluorescein was irreversible even after rehydration. However when stored, the entrapped antibodies at 4°C in water, is stable up to 4 weeks. In another report (148) antifluorescin antibody was entrapped in sandwich configuration, in aerosol-generated sol–gel-derived thin films ($0.62 \pm 0.05\ \mu$m). The fluorescent hapten 5-(and 6-) carboxy-4′5-dimethylfluorescein (Me_2F) was employed to determine the accessibility and viability of the entrapped antifluorescein antibody. Antibodies were efficient in recognition and binding of the Me_2F up to 13 weeks when stored in 0.1 M pH 8 PBS at 4°C. For the regeneration, antibodies were treated with a mild chaotrope i.e., 4 M NaCl, with which 40–50% of the regeneration was achieved, up to 6 cycles (143). Yang et al. (320) reported that the addition of PEG did not affect the encapsulation efficiencies of the antigentamicin antibody. But the encapsulation efficiencies of the antibody in two kinds of sol–gel materials i.e., with and without PEG were 93 and 91%, respectively. But the addition of PEG showed a strong effect on the binding activity of the encapsulated antibody. When the antibody encapsulated in sol–gel along with PEG, 95% of the gentamicin was bound to the column, in case of without PEG only 42% bound to the column. This improved binding activities probably due to the high diffusion rate of the gentamicin. Furthermore, addition of PEG prevented the shrinking of the sol–gel glass matrix and denaturation of the immobilized antibodies. Similarly, other studies also reported that the addtion of PEG stabilized the AChE and urease and prevented the fouling and adhesion of unwanted protein to the surface (321, 322). Hence while designing the matrices for immobilization of biomolecules, it is imperative to choose dimensions of the support matrices so as to minimize diffusional resistance, thereby making the entire population of encapsulated biomolecules participate in reaction. Hydatidosis is a disease, which was caused by *Echinococcus granulosus* tapeworm transmits through the dogs and sometimes through the sheep, cattle and pigs. This disease causes cysts on the lungs, liver and other organs in the human body and easily infects other organs and causes echinoccosis. Hydatidosisis antigens have been isolated from the cysts, immobilized in the TMOS sol–gel, and coated into ELISA plate wells for the detection of disease. The antigens entrapped in the sol–gel matrix retain biological activity, and antibodies in human sera are able to diffuse thorough the pores of the silica matrix where they react specifically with antigens (323). sol–gel matrices and normal ELISA optical density measurements are comparable, it indicates that under controlled pore size (high porosity 50 Å) antibodies can easily diffuse through the sol–gel and can react with antibody as in solution.

Shabat et al. (324) successfully encapsulated catalytic antiboides through sol–gel method and used them in the transformation reactions. Antibody 14D9 is an effective catalyst for various hydrolytic reactions including the hydrolysis of a cyclic acetal, ketals, epoxides and enolethors. The catalyst has been homogeneously doped inside the gel matrix and shows a catalysis followed Michaelis–Menten kinetics with a slight increase in K_m, attributed to the hindrance of reorientational motions near the active site. sol–gel encapsulated anti-TNT antibody has been used as detector for TNT (325). The entrapped antibody is more stable than that immobilized through surface attachment. It is able to differentiate TNT and analogue trinitrobenzene (TNB), and can be employed in different immunoassay formats such as competitive and displacement assays. The aged gels exhibit significantly faster response than xerogels due to the larger pore diameter. Tofiño et al. (326) developed a flow-through fluoroimmunosensor for the determination of isoproturon. The encapsulated antibodies were completely retained the activity and exhibited good performance in competitive immunoassay. The FITC labeled and free isoproturon diffused through the pores of sol–gel and bound with antibody without any nonspecific interaction. Detection limit for the immunosensor was $2\ \mu g\ L^{-1}$ with a dynamic range of 0.002 to 0.2 and 0.004 to 1 mg L^{-1}, depending on the immobilized antibody concentration. Real samples, herbicide spiked potato samples and spiked seawater, were quantified using this sensor. The antigentamicin Mab entrapped in mesoporous TMOS so-gel monolith was employed for the development of flow injection fluorescence immunoassay for the quantitative analysis of the gentamicn (320). Gentamicin is a broadspectrum antibiotic and at higher dosages it causes impaired renal function, hence accurate monitoring of the drug in serum of patients is mandatory. Entapped antigentamicin MAbs were able to detect antibiotic upto 200 ng ml^{-1} in the serum with a working range of 250–5000 ng ml^{-1}. The antibodies (immunoreactor) could be reused up to 20 cycles using 5 mM NaOH as an elution buffer. Similarly when employed pH 2.5

glycine-HCl buffer, the regeneration efficiency was good, but fluorescence intensity of FITC (gentamicin-FITC conjugate) reduced. Immunoreactor column was stable to up to 1 month at room temperature and 3 months at 4°C (320). Hormone (cortisol) determination through sol–gel entrapped antibody was reported recently (42). Anticortisol antibodies were encapsulated in optically transparent sol–gel silica matrices and competitive immunoassays for cortisol were conducted using the antibody-doped silica material as sensing elements. Between the monolith and thin-film, thin films showed good accessibility of antigen to the encapsulated antibody and significant reduction in assay time. The encapsulated antibodies were able to detect cortisol in the range of 1–100 μg dl^{-1} with controlled non-specific binding to the sol–gel silica matrix. More importantly the encapsulated antibody fluorescence signal was 10 times higher than the surface-adsorbed antibody. It was attributed to the possibility of more antibody encasulation per unit volume of sol–gel (42).

Wang et al. (327) reported sol–gel derived thick-film amperometric immunosensor, by encapsulation of antigen (RIgG) in TEOS sol-gels. This thick-film immunosensor showed very fast response (20 s) due to the effective contact between electrode surface and reaction centers though the doped graphite powder, retained antigenic properties of the sol–gel entrapped antigen and effective binding efficiency between antigen and anti IgG-HRP conjugate. One of the major obstacles in practical use of the immunosensing devices is the difficulty associated with the regeneration of the sensing surface. Because the binding force between antigen and antibody is relatively strong, involved biomolecules are fragile and sensitive. In view of this few of the researchers has developed renewable electrode surfaces by immobilizing the antigen into the sol–gel matrices. *Schistosoma japonicum* antigens (SjAg) were immobilized in TMOS along with BSA and graphite powder, finally squeezed into the PVC tube with screw of thread at one end. Through screwing up and polishing easy to regenerate the electrode surface (328). Using competitive immunoassay between HRP-SjAb, SjAb and SjAg entrapped on the electrode surface, the immunosensor was able to detect SjAb up to 4.5 ng mL^{-1} through fluorescence detection. More recently Zhong and Liu (329) reported another sol–gel amperometric immunosensor for *Schistosoma japonicum* antibody assay using the same immobilization procedure by doping with BSA and graphite. The presence of BSA reduced the nonspecific binding of SjAb/SjAb-HRP conjugate to the electrode surface. The doping of graphite served as a conductor to complete the circuit. The developed immunosensor showed only 10% reduction in amperometric signal even after 1 month and it could be used up to 100 immunoassays. In another study (330) complement 3 (C3) antibody was immobilized in the methyltrimethoxysilane (MTMOS), BSA and graphite mixture to construct a renewable amperometric immunosensor for determination of complement 3 in the serum. In this study competitive immunoassay was carried out between sol–gel encapsulated antibody, C3 antigen and C3-HRP conjugate. Immunosensor had a linear range between 1.17–35.1 μg mL^{-1} with a detection limit of 0.56 μg mL^{-1}. Gong et al. (331) designed an amperometric immunosensor by encapsulating the Newcastle disease antigen (NDAg) in TEOS sol–gel. In this investigation antigen was entrapped in two different matrices i.e., paraffin-ND Ag-BSA and sol–gel-ND Ag-BSA. sol–gel-NDAg-BSA had a good sensitivity in determination of NDAb present in the rabbit serum, and the antigen retained antigenic properties very effectively in sol–gel.

Immunosorbents. The accurate determination and the development of new diagnostic immunoassays for pesticide residues and environmental contaminants require the development and the establishment of simple extraction, cleanup, and concentration procedures. In recent years the development of new adsorbent materials and better methodologies for the solid phase extraction (SPE) of trace pollutants from environmental matrixes have been the subject of much research work. Because of their high selectivity, immunosorbents have been becoming one of the most interesting new SPE tools for environmental analysis. Affinity purification method has been widely employed for the purification or various compounds successfully, by attaching the recognition agents on to the solid surface. The sol–gel entrapment method facilitates more simple, low-cost, quick and one-step entrapment of either IgGs or whole antiserum and no need of any preliminary Ab purification (48, 49). Hence sol–gel entrapped Abs employed in affinity purification by packing into column (immunosorbent). However the first step in the development of an IAP procedure is the determination of optimal conditions for Ab immobilization. The successful entrapment of dopants requires substantial screening of the sol–gel preparation procedure parameters, including examination of the effects of sol–gel format and composition on binding (31, 116). Altstein et al. (332) immobilized TNT IgG antibodies in TMOS monoliths. The resulted wet gels were thoroughly crushed and packed into the 1 ml columns for the separation and quantification of TNT. The entrapped antibodies exhibited dose dependence, highly reproducible binding, and offered capacities higher than or comparable with those of protein-A-agarose-coupled Abs, and allowed simple, quick, and high-recovery elution of the analyte (100–88% for 10 and 90 ng mL^{-1} TNT respectively), with almost no leaching of the Ab or analyte. Lira et al. (333) reported 2,4-dichlorophenoxyacetic acid (2,4-D) immunosorbent for the extraction of 2,4-D ester. The immunosorbent showed good precision, no leakage of the antibody and a binding capacity of 130 ng of 2,4-D ester per mg of immobilized antibody, corresponding to 42% of the free antibody activity. Using 50% V/V acetonitrile in phosphate buffer a good recovery of the 2,4-D ester from the immunosorbent was obtained.

Bronshtein et al. (48) reported that the presence of non-relevant proteins along with atrazine MAb in sol–gel did not have any effect on specific and non-specific binding of antibodies. It indicates that the purification of MAbs from hybridoma culture fluids is not necessary for the immunosorbent's preparation

when employing the sol–gel immobilization process. However the presence of relatively large amount of proteins in the sol–gel may has an impact on the reproducibility of the systems. Unpurified hybridoma culture fluids showed 16–45% of variabilities in sol–gel experiments performed with MAb, against to a variation of 2–6% in experiments performed with IgGs. Then, 500 μL MAb (2.5 mg protein/ml of sol–gel) entrapped in TMOS (TMOS:aqueous = 1:8, doped with 10% (v/v) PEG) bound with 45 ng of atrazine within 15 minutes at room temperature. The entrapped MAb was stable up to 53 days at room temperature and did not display any decrease in binding capacity. But when kept in solution (with 0.01% thimerosal) at room temperature in a sealed tube for 36 and 55 days, 40 and 60% of their activity lost, respectively, compared with those that were kept in solution at 4°C. The elution of antigen from entrapped antibody is another crucial factor in immunosorbent development, in this study 69% and 86 % of atrazine recovery was reported when employing 0.1 M pH 3.5 glycine buffer and 0.1 M pH 11.5 triethylamine buffer, respectively. In another report Bronshtein et al. (49) developed an immunosorbent for the extraction of nitroaromatic compounds from the aqueous solution by the systematic optimization of various important parameters. At the optimized conditions 87% of 2,4-dinitrophenylhydrazine (DNPH) was bound with the encapsulated antibody. Compared with xerogels, wet gels show good binding efficiency and porosity. The dry gels result in conformational changes of antibody due to the drastic change in pH and reduced porosity during the sol–gel process. Whereas in the case of wet gels, these side effects can be minimized and antibodies are always exposed to the aqueous environment, hence retain good binding efficiency towards antigen. The doping of PEG improved the binding efficiencey, probably due to higher number of pores, and protective matrix than ordinary silicates. The most important finding in this report is the presence of non-specific proteins (serum) does not show any effect on binding affinity of antibody. During Ab purification process the loss of antibody activity occurs at the elution steps, this can be avoided by direct encapsulation of the antibody in serum. Hence low amount of IgG in serum is enough for immunosorbent compared to the purified antibody, which is a great advantage with sol–gel encapsulation. Similarly the presence of non-specific proteins does not impaire the binding efficiency of sol–gel entrapped atrazine MAbs, interestingly they does not provide any protective effect on Abs (48). It indicates that while entrapping the biomolecules, their vicinity should be closely monitored. DNPH is eluted from entrapped antibody using glycine–HCl (pH 3.5) and absolute ethanol with the recoveries of 69 and 91%, respectively. The above studies demonstrate that in the future, sol–gels, entrapped immunoglobulins can be effectively employed in the development of biosensors and immunosorbents in simple, quite cheaper manner. sol–gel immunosorbents will become a potential affinity sorption tools for the extraction of toxins, pollutants and other proteins from the environmental and biological complex mixtures.

Whole Cell Encapsulation

Recent progresses in cell culture and microfabrication technologies have stressed the development of novel immobilization techniques for whole cell stabilization. The development of cell-based biosensors is highly useful for the functional characterization and detection of various compounds such as drugs, toxins, and drug testing. The industrial development of biotechnologies is another important applied area in the search for new immobilization procedures. They need the immobilization of active biospecies such as whole cells on the solid substrates with restricted growth and mechanical stability. The major advantages with whole cell immobilization are that it can act as a bag of enzymes, and the protein conformation is more stable, as it was in native environment. At the same time this technique can reduce the purification costs and easy of encapsulation. The encapsulated dead biomass can be used as biosorbents for the removal of toxic metals. The functionality of the emerging class of live-cell ceramic hybrids is largely dependent on the way. The encapsulating host influences the cells' physiology, which arouses new challenges (29).

Microorganisms. The wild, mutant and recombinant strains are widely employed for the production of several commercially important metabolites. Few of the immobilized strains have been employed for the commercial industrial applications. Alcohol is generally used as a disinfactant, as it solubilizes microbial cell walls, which causes the death. To improve the alcohol tolerence limit genetically engineered strains are developed. The physical caging in sol–gel is another possible way to improve the alcohol tolerance. Recently Desimone et al. (320) immobilized *Saccharomyces cerevisiae* cells in sol–gel films. These cells exhibited improved tolerance limits to ethanol, 1-propanol, 1-butanol, 1-pentanol and 1-octonal. The tolerance was due to the tight binding of the water to silica matrices, it helped in protecting the cell against the water distorting activity of the surrounding organic solvent (334). Like proteins, entrapped microbes also showed random distribution in sol–gels without any aggregation. The enzymatic activity decreased rapidly when the gel was dried under ambient conditions compared to freeze-drying. Immobilized *E.coli* cells followed the Michaelis-Menten equation when quantified the β-glucosidase activity via the hydrolysis of 4-nitrophenyl-β-D-galactopyranosdie (4-NPG) (335). It indicated that even though enzymes were embedded in the native environments (inside the cell), the sol–gel conditions should be controlled for effeicent conversions of enzymes.

Chia et al. (45) reported that sol–gel-encapsulated yeast cells were live even after dip-coating process and both fresh and dry films that were stored in air at room temperature for up to 3 days. Silicate film and entrapped cells were accessible to the substrate and fluorescent molecules, which was demonstrated through the fluorescent studies by dipping the entrapped cells into 4′,6-diamidino-2-phenylindole (DAPI) solution (DAPI is a DNA specific fluorescent molecule). DAPI diffused through the sol–gel and cell membranes specifically bound with DNA, which led to 20% increase in the fluorescence. The entrapped

cells could be employed to screen libraries based on color or fluorescence. For example, a library of coding sequence representing an entire gene fused to green fluorescent protein (GFP) could be screened by fluorescence. To study the bacterial growth and to improve the cell viability Conffier et al. (336) immobilized yeast cells through aqueous sol–gel route. The measurement of the β-glucosidase activity showed that aqueous sol–gel process was less detrimental to cell viability than usual alkoxide route. Moreover, the aqueous silica matrix appeared to slow down the lysis of cell membrane when bacteria were aged without nutrients, but some lysis occurred during the sol–gel encapsulation. In another study Nassif et al. (337) doped gelatin into aqueous silica sol–gel along with bacterial cells to study the cell viability. The doping of gelatin prevented the cell lysis, which usually occurs during the initial gelation process. More recently recombinant green and red fluorescent bacteria were encapsulated in sol–gel derived silica films. The viability, leaching and distribution of cells in sol–gel were studied by measuring the green and red fluorescent protein expression in presence of inducers such as mitomycin C (MMC) and nalidixic acid (NA) (338). The encapsulated *E.coli* cells maintained long-term viability without any leakage of cells. Even dead cells also showed fluorescence, due to the perfect caging nature of sol–gel. In addition, the cell proliferation was also controlled by the sol–gel cage, which prevented the contamination from other contaminant bacterial species. Because the pore size could be completely controlled, otherwise the entrapped cell cannot leach out. Confocal microscopic studies enlightened that the cells were homogeneously distributed in the silicate film, the distribution was not uniform, some locations contained a single bacterium per cage and cells were embedded even deep inside the film (338). Ferrer et al. (339) studied the effect of methanol, which was formed during the hydrolysis and condensation (sol–gel process), on the cell viability and its functionality. In this study genetically engineered *E. coli* TG1/pPBG11 strain, which can express a green fluorescent protein (GFP) in response to the presence of alkanes, or dicyclopropyl ketone (DCPK) was used. Above $\sim$1.2 M methanol disrupted completely the cell and decreased fluorescence intensity. It was attributed to the unfolding of the protein and fast internal conversion. However the organically modified silica matrixes, an alcohol free process, improved the cell integrity up to 95%. Chen et al. (340) entrapped *Methylomonas Sps* strain GYJ3 in sodium silicate and methyl trimethoxysilane (MTMS) sol-gels. The activity of free and immobilized cells was determined by the rate of propylene epoxidation to proplylene oxide. The cells entrapped in sodium silicate gel matrix exhibited about 1.5-fold higher activity than the free cells. Whereas in case of MTMS lower activities were reported due to the formation of alcohol during the gelation, which had negative effect on microbial cells. Bhatia and Brinker (66) reported the similar kind of negative phenomena in case of lipase entrapped in a orthosilicate-based matrix, they found only 5% lipase activity after entrapment. It indicated that the alcohol absence favored maintenance of the integrity of the cell and thus the proper response of the cell to external signals. For the construction of whole cell based biosensors cell response should be quicker and highly sensitive even at lower concentrations of inducer/analyte. Recombinant cells were comparatively more sensitive than the wild strains.

The most important factor in construction of whole cells sensors is the immobilization of cells. Premkumar et al. (341) immobilized recombinant luminous bacteria into TEOS sol–gel to study the effect of sol–gel conditions on the cell response (luminescence). The entrapped and free cells showed almost the same intensity of luminescence (little lower), but the entrapped cells were long-term stable than the free cells (4 weeks at 4°C). It revealed that the encapsulated recombinant bacteria maintained their physiological ability to synthesize a fully functional luciferase and sufficient substrate to generate luminescence. Green fluorescent protein expressing *E.coli (pET-gfp)* dopped in the aerosols expressed the protein after exposed to the bacteriophase. The induction of GFP indicated that both bacteriophase and *E.coli* survived the stressful desiccating conditions of the aerosol challenge and the pore size was suitable to the diffusion of the bacteria phase. This entrapped microorganism could be used as recognition agents for the detection of toxic chemicals and pathogenic microrganisms (342). Carturan et al. (14) encapsulated *Saccharomyces cerevisiae* (yeast cells) in thin sol–gel films on activated soda lime glass sheets. The activation energy of the immobilized and free cells were the same, but the catalytic activity (invertase) decreased, though K_m (immobilized) was smaller than that of free cells. It was reasoned that the chemical modification of the biocatalyst, involving the interaction of cell membrane with the Si-OH groups. They observed cell aggregation inside the gel layer due to the reduced dispersion of the yeast cells. Betancur et al. (343) encapsulated *Lactobacillus helveticus* in TEOS. The viability of the cells was monitored through the conversion capacity of the lactose to lactic acid. The matrix showed good mechanical resistance and stability, and the pore size was not restrictive to the transfer of molecules i.e., medium components and product. All these helped in the improved conversion capacity of cell. The immobilized cells could be repeatedly used for five successive fermentations of 40 hours each cycle.

Sol–Gel Biosorbents and Biocers. Microorganisms have been employed for the bioremediation and bioaccumulation of environmentally hazardous compounds. Recently the sol–gel technique has been used to accumulate metal ions by entrapping the cell/spores/cell wall proteins. The yeast cell walls isolated from the waste breweries biomass were entrapped into TEOS sol–gel and used as biosorbents for the removal of Cd^{2+} and Ag^{+} from the aqueous samples. The biosorbent showed better mechanical strength and sorption capacity than the cross-linked with epichlorohydrin (344). *Bacillus sphaericus* JG-A12 isolated from the uranium mining water were entrapped in aqueous silica nanosol for the accumulation of copper and uranium. The entrapped cells exhibited more sorption capacity than the spores and S-layer (protein present on the cells wall and acts as

a molecular sieve and trap). The doping of sorbitol and freeze-drying of sol–gel improved the metal binding capacity due to the improvement of pore size and less shrinkage (345, 346). The *Bacillus sphaericus* entrapped in pure silicate matrix bound less uranium and no copper. Both metals were completely removed from the free and entrapped cells using aqueous citric acid to reuse the biosorbent. One way of protecting the environment from pollutants is biodegradation. Phenol is one of the most potent toxic compounds in water bodies. For the degradation of phenol the free and immobilized microbes were employed in several studies.

By combining the silica sols with alumina fibers new forms of biocers (silica and ceramic materials with embedded living cells-Biocer) were developed by Fielder et al. (347). In biocers *Rhodococcus rhodochrous* cells and *Aspergillus versicolor* spores were entrapped and used for degradation of phenol and glycerol. The cells embedded in (alumina fibers reinforced) aqueous sol–gels showed high biocatalytic activity, high compactness, and less shrinkage during the drying. In addtion, this doping of glycerol fiber reinforced biocers improved the biocatalytic activity, as polyols (glycerol, PVA) could act as a matrix softener and humidity retainer, which led to suppress in cell lysis and extended the viability of the entrapped cells. Sol–gel encapsulated microorganisms performed good response to the nutrients and metabolic activates like free cells in broth. The encapsulated sulfate reducing bacteria reduced the sulfate ions at the rate of $\sim 11\ \mu g\ h^{-1}\ cm^{-3}$ gel and was stable up to 10 weeks. At more prolonged times, initial reducing activities were decreased, but when exposed the cells to nutrient medium again, cells growth took place and activities were improved, like non-immobilized cells (348). These studies revealed that the entrapped cells were viable within the sol–gel. Due to the good diffusional properties of the sol-gels, microorganisms were enable to respond to the nutrients and inducers along with improved stability in terms long-term activity.

In biodegradation and conversion process using immobilized cells, controlled growth of microorganisms is necessary. Overgrowth leads to the leakage of biomass and resticted transfer of metabolites and analytes into the cells. Branyik et al. (349) carried out comparision studies to investigate the influence of immobilization on cells growth and their degration capacity to phenol and polychlorinated biphenyls (PCBs). Lower biodegradation capacity was found in case of sol–gel immobilized cells, but it well controlled the excess growth and leakage along with lower swelling, whereas polyurethane foam was swelled 6 times more than TEOS and cell leakage was also reported. Hence these non-swelling and growth restction features were useful in fermentation process. By combining novel matrices, improved sol–gel process and the advantageous features of sol–gels, in future cell conversions, capacities could be improved.

Plant Cells. Compared to enzymes, sol–gel encapsulation of microbes has less been done. Similarly compared with the research of microbes animal and plant cells is almost at juvenile stage because these are comparatively bigger in size, fragile and need highly controlled environments. Hence the direct use of normal alkoxide sol–gel procedures is not applicable for the plant/animal cells like other proteins, and monoliths cannot be ground into small particles. To circumvent this, a novel biosil method has been introduced, in which sol–gel siliceous layers were deposited through airflow on to the cell surface, which encapsulated whole cell. This procedure has been described in earlier reports (28, 43, 44, 80, 98, 350), for the convenience of readers, the process outlined by Boninsegna et al. (44) is given here once again and schematic diagram is shown in Figure 4.

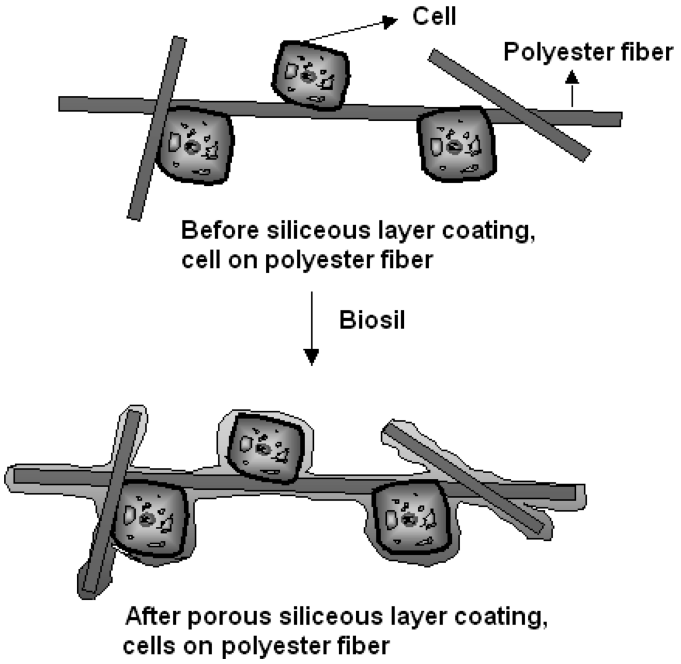

FIG. 4. Schematic diagram for cells on polyester fibers encapsulated by porous siliceous layer.

1. Silicon alkoxides in gaseous phase are fluxed by an inert gas carrier at the surface of cells adhering to a scaffolding material.
2. Reaction with water sorbed on the cell surface or with exposed hydroxides affords solid siliceous products, with concomitant removal of toxic alcoholic by-products by the gas carrier.
3. Siliceous membrane thickness may be controlled by exposure time and does not alter original cell morphology. The membrane is homogeneous, since it is build up by a continuous gas supply directly investing the cell surface.
4. Membrane porosity and mechanical stability may be adjusted by the correct choice of silicon alkoxide precursors, as demonstrated for bulk materials obtained from the same precursors.

Few of the plant cells were successfully immobilized using biosil process without any significant negative effects on cells and leakage. Invertase is a complex enzyme, commercially

produced through bacterial fermentation. The disadvantage associated with bacterial invertase is sometimes contaminated with noxious proteins and presence of prions. The potential alternative for this is production of invertase through the plant cells. Pressi et al. (82) immobilized *Ajuga reptans* AYN-IRB1 plant cells by biosil method for the invertase production. *Ajuga reptans cells* were isolated from the callus, which was propagated thorough the plant tissue culture and subjected into growth medium. During the second day of growth, fiber disks were added into the cell suspension for the anchorage. After growth for 7 days, the disks were washed and exposed to an air flow saturated by a 60/30/10 volumetric mixture of $Si(OEt)_4$, $CH_3Si(OEt)_3$ and $CH_3SiH(OEt)_2$ for 20 minutes ensuring the deposition of siliceous layer on the cell surface. The resulted encapsulated cells were viable up to 70–75%. Encasulation prevented the cell leakage and suppressed the cell repoduction. About a 40-fold increase in invertase production was reported using biosil encapsulated plant cells. Hata et al. (351) immobilized photosynthetic pigment chlorophyll *a* into silica surfactant nanocomposites films. Chlorophyll *a* was stable against visible light in comparison with a homogeneous chlorophyll *a* solution system even under an air atmosphere. In another study Carturan et al. (13) immobilized *Catharanthus roseus* plant cells through biosil method for the production of alkaloids vincristin and vinblastin. Most interestingly the immobilized cells were stable up to 100 days with continuos production of alkolid. The productivity of encapsulated cells is two orders of magnitude higher than free cells.

Animal Cells. One of the major challenges of microencapsulation techniques is to immobilize living cells behind a semi permeable membrane thereby allowing their transplantation. In recent years transplantation therapy has emerged as a promising potential treatment strategy for curing diseases. For the efficicent trnasplantation the matrix used for the encapsulation should have good permeability, which allows the diffusion of small substrates and products, should avoid diffusion of immune system agents, high mechanical strength and non-immunorejection (352). The silica and sol–gel materials are biocompatible (nonimmunorejection). Mechanical features of sol–gel SiO_2 coatings in presence of entrapped animal cells was studied by Sglavo et al. (350). In view of the nonimmunorejection and stability of the sol–gel films pancreatic islets, which can produce insulin, were immobilized thorough biosil process (353). The encapsulated cells were able to produce insulin up to 3 weeks in vitro conditions. For the in vivo studies encapsulated islets were transplanted peritoneally in several non-obese diabetic mice. After transplantation of islets urinary excretion of glucose fell to almost zero with in few days. With similar way Boninsegna et al. (44) immobilized rat pancreatic islets in siliceous layer and transplanted them into diabetic rats. The encapsulated islets showed long-term constant levels of glycemia with about immunorejection compared with nonencasluated islets.

Several proteins, plant and animal cells have been immobilized in calcium alginate beads, however the major drawback in this method is instability of beads in presence of citrate ions and easy disintegration at simple shear stress. Boninsegna et al. (81) developed alginate silica microcapsules.

In this procedure animal cells or cell aggregates were suspended in Na-alginate solution and extruded under a flow of air saturated with silicon alkoxides. The immediate formation of a sol–gel siliceous membrane on the microdrop surface allowed the reaction with Ca^{2+}, leading to solid Ca-alginate and yielding homogeneous, stable microcapsules of 200 μm in diameter. In this method the alginate droplet size was controlled by flowing air at the tip of the extrusion nozzle. Human hepatoblastoma (HepG2) and human T leukaemia Jurkat cells have been encapsulated in alginate silica microsperes for the production of albumin (HSA) human interleukin 2 (IL2), respectively. The important findings in this study were outer siliceous layer acted as a physical barrier and provided more protective cage to calcium-alginate microcapsules. The siliceous layer was stable and preserved the entrapped cells from leakage. Perhaps non-coated alginate microcapsules were rapidly dissolved. The siliceous coating on capsules did not interfere with bioactivity of the cells or diffusion of the products, it only provided mechanical and chemical stability to the calcium-alginate microcapsules (52).

CONCLUSIONS

The functionality of immobilized biomolecules is governed mainly by the nature of the biomolecule, the preparation method, as well as the nature and structure of the immobilisation matrix. Among the most important properties of the encapsulated enzymes, good accessibility to analytes and long-term stability for both operation and storage are prime essentials. Many kinds of bio-components have been preferentially immobilized by both neat and hybrid silicate gel-processing methods to develop sol–gel-derived biomaterials, and this should be intensified in the future. While most sol–gel bioencapsulates reported to date have used inorganic materials or carbon composite derived from either tetramethylorthosilicate (TMOS) or tetraethylorthosilicate (TEOS). Even though some other precursors have been introduced, still newer more milder sol–gel derived materials are highly desired for improving the analytical capabilities of biosensing devices, and for meeting the need of practical application and commercial production. Maybe the new hybrid sol–gel materials and/or methods could provide more electrochemically active, less brittle nature and biocompatible matrices. For the long-term use and reduction of film fouling though microbial growth or adhesion, new matrices that can inhibit the growth have to be explored. This can be possible by developing the new sol–gel precursor molecules, which are modified with microbial inhibition compounds or metal ions.

Even though the plant and animal cells and spores of microbes can be immobilized in silica matrixes, still the main drawback is their reduced viability. Towards this direction more basic studies are needed for the potential use of sol–gel advantages and for the industrial/biotechnological application of cells. More mild,

stable gelation conditions should be developed to improve the cells' viability. However, the studies such as interactions between the sol–gel matrix and whole cells and metabolic changes duing immobilization have to be closely monitorrd for the exploration of new matrices and methods. Such new sol–gel methods/matrices can improve the productivities and be used to design the novel whole cell biosensors and spwan the medical applications. Furthermore cells/proteins immobilized in sol–gel matices can be effectively applied in biochemistry, biotechnology, environmental monitoring, instrumentation, forensic science, laboratory automation and pharmaceutical chemistry for multiple applications with practical significance in near future.

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