Bio-doped Nanocomposite Polymers: Sol−Gel Bioencapsulates

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The period from 1970s to 1980s witnessed notable interdisciplinary breakthroughs in sol−gel science with demonstrations that this technology could be extended to the encapsulation of functional biomolecules such as enzymes and antibodies within ceramic matrixes. Since these landmark studies, some of nature's most sensitive biological materials, including proteins, DNA, RNA, and antigens as well as more complex assemblages such as cell membranes and organelles, and even living microbial, plant, and animal cells, have been entrapped in inorganic and inorganic−organic hybrid sol−gel polymers. Bioencapsulation retains not only the structural integrity of the entrapped biomolecules but also, more importantly, their full biological functioning—from molecular recognition, catalysis, and signal transduction to sustained cell metabolism and reproduction. The ability to marry the physicochemical features of inorganic, hybrid, and composite polymers with the selective binding, catalytic, and biosynthetic functions of biological materials has enabled the fabrication of novel high-performance bioactive nanocomposites for sensor, catalyst, diagnostic, and electronics applications.

Introduction

A century of sol−gel science has seen its founding with the discovery of room-temperature hydrolytic routes to silica glasses and its diversification into technologies for the preparation of highly structured nano- to macroporous inorganic and hybrid organic−inorganic polymers with an astounding range of chemistries.[1−6] Silicas, main group and transition metal oxides, metallosilicates, organically modified silicates (Ormosils),[1−4] and various composites,[2,5−7] have been synthesized for such applications as optics, energy storage, memory devices, and sensors. The advent of structure-templating[5] and precision fabrication methods has further advanced sol−gel applications in separations science,[7] heterogeneous catalysis,[8,9] and microdevice technology.

A prominent feature of sol−gel technology that was recognized at an early stage is that small molecules, such as transition metal complexes and organic dyes, can be encapsulated with sol−gels to form doped polymers. Furthermore, the entrapped molecules largely retain their native chemical characteristics within the polymers, and this together with the unique combination of optical transparency and porosity to low-molecular-weight molecules enable their use as optical materials, chemical sensors, catalysts, and so forth.[1−9] Given the aqueous chemistry of sol−gel processing and the unique access to room-temperature ceramics synthesis, the technology was an obvious candidate for interdisciplinary extension into the realm of biology. One can ultimately trace sol−gel bioencapsulation back to 1955 and the fortuitous work of F. H. Dickey on the imprinting of silicic-acid-derived xerogels with organic species with the aim of producing specific adsorbents.[10] Failing in his efforts to make selective adsorbents, Dickey observed that organic dyes were strongly retained and therein stabilized when encapsulated in silica xerogels. Dickey went on to demonstrate that the proteins urease, catalase, adenylate deaminase, and cytochrome *c* could similarly be immobilized within silicas wherein they were highly resistant to leaching and, more remarkably, that they retained varying degrees of their native enzymatic and redox activities. Although Dickey postulated that the silica-encapsulated proteins could be used as stabilized reagents, he failed to realize the true promise of his observations, and the potential of his results were overlooked. The first in-depth exploration of silica-encapsulated proteins followed in 1971 with Johnson and Whatley's investigation into the catalytic properties of trypsin encapsulated in silicic acid xerogels. These authors disclosed that trypsin retained up to 34% of its solution activity upon encapsulation, that the enzymology of the entrapped enzyme mirrored that of the soluble protein, and that encapsulation considerably enhanced stability.[11] Once again, the larger ramifications of the results were missed, and no further work in the field was recorded. It is important to note that these two studies utilized colloidal silicic acids (formed from the acidification of aqueous sodium silicates) containing preformed tetrahedral SiO_4 units, as the precursors for silica hydrogel/xerogel formation. It is now felt that true sol−gel processes should be restricted to those based upon silicon alkoxide precursors, the hydrolysis and condensation of which lead to the formation of polymeric silicates that are distinct

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from silicic-acid-derived materials. Strictly speaking, the aforementioned studies may not be true examples of sol−gel bioencapsulation, but their precedence and relevance for the field is clear.

It was not until the mid 1980s that the critical advance of recognizing that biologicals can be encapsulated via silicon alkoxide sol−gel chemistry was made.[12,13] Thus, Venton et al. showed that anti-progesterone antibodies could be immobilized within silica-poly(3-aminopropylsiloxane) xerogels and that the entrapped proteins retained their highly selective binding for progesterone.[12] Later, Glad et al. demonstrated that the enzymes glucose oxidase, horseradish peroxidase, trypsin, and alkaline phosphatase could be entrapped in monolithic and thick film silica-poly[*N*,*N*-bis(2'-hydroxyethyl)-3-aminopropylsiloxane) sol−gels and the resulting nanocomposites efficiently catalyzed oxidation and hydrolysis reactions as per the native enzymes.[13] Significantly, these groups utilized aminoalkyl- and aminohydroxyalkyl-substituted alkoxysilanes rather than tetraalkoxysilanes as precursors, to form organically modified silicates (Ormosils) rather than pure silicas. This was derived from the view that alkoxysilanes bearing functional organic ligands were requisite for efficient bioencapsulation and that simple precursors such as tetramethoxysilane (TMOS) and tetraethoxysilane (TEOS) were not suitable for this purpose.

Notwithstanding the above works, the crucial advances in sol−gel bioencapsulation which led to the recognition and establishment of the field and provided the impetus for the extensive investigations which have followed since came from the pioneering efforts of Avnir and co-workers.[14] In 1990, this team disclosed that proteins can be encapsulated using facile sol−gel protocols based upon TMOS and TEOS precursors, as demostrated with the preparation of catalytically active and stable, transparent silica xerogels doped with the enzymes alkaline phosphatase, chitinase, aspartase, and β-glucosidase. This was followed in 1992 with the work of Ellerby et al., who used this methodology to entrap the metalloproteins copper−zinc superoxide dismutase, cytochrome *c*, and myoglobin in silica sol−gels.[15] Confirming the findings of Avnir et al., they showed that the protein−silica nanocomposites displayed the typical catalytic, metal-exchange, oxidation−reduction, and ligand-binding reactions of the soluble proteins.

These founding publications firmly established that labile biological molecules with catalytic, recognition, and transduction functions could be incorporated into sol−gel materials with minor modifications in protocols.[16−29] Numerous works have since shown that the technique is generic and can accommodate a wide variety of labile biological materials.[16−29] It has also become apparent that sol−gel chemistry is not unique in this respect—conventional condensation and addition polymers such as polyacrylates, polystyrenes, and silicones can also be manipulated to encapsulate native, surfactant-complexed, and chemically modified proteins.[30] To date, a host of natural and engineered proteins, polypeptides, antibodies, antigens, DNA, RNA, cell membrane fractions, organelles, and whole cells have been encapsulated in a diverse range of inorganic, organic, and hybrid polymers. Although research has centered on developing highly selective and sensitive biosensors for industrial, medical, and environmental analysis,[22−28] interest has also turned to catalyst and bioelectronics applications.[16,29,30]

General Considerations for the Encapsulation of Biomolecular Structures

Before delving into the realm of sol−gel bioencapsulation, it is useful to give an overview of some pertinent features of proteins:[31−34]

(a) Typical proteins are linear polymers with molecular weights of approximately 5000−100 000 for monomers to over 400 000 for oligomeric structures,[31] corresponding to sizes of approximately 1−6 nm for globular conformations, although oligomers and membrane proteins may have much larger dimensions along their major axes. Thus, most proteins are in the pore size range of mesoporous materials.[16−21]

(b) The constituent amino acids of proteins possess neutral, hydrophilic, hydrophobic, basic, and acidic functionalities, which can be organized at the primary, secondary, and/or tertiary level to form ordered displays which are often critical to binding, catalysis, and/or stability.[31−34] pH, solutes, solvents, and liquid or solid interfaces that interact (hydrophobic, ionic, H-bonding, etc.) with such surfaces can influence the ionization state, hydration and hydrophobicity, dynamics, and ligand partitioning and have an impact on biomolecule structure, solubility, aggregation, and activity.[31, 33,34]

(c) Proteins are hierarchical: the primary structure (amino acid sequence) is ordered into a secondary structure (β-sheets, α-helices, etc.), which is spatially organized into a (monomeric) tertiary structure (α−α packed helices, β−α−β barrels, etc.) which may associate with other monomers to form homo- or hetero-oligomeric assemblies (cylinders, toroids, sheets, etc.) that comprise quaternary structures.[31,32] Critical to recognition and catalysis is the binding site, a specific arrangement of amino acids, as well as metal centers or photo-/redox-active cofactors in more complex proteins. The active site constituents interact among themselves as well as surrounding protein residues and water molecules in a precise fashion to create a topologically and physicochemically defined chiral microenvironment, the structure and dynamics of which dictate the selectivity and efficiency of recognition and catalysis.[31,32] Thus, the maintenance of short- and long-range structural order is critical to protein function, and pH, solutes, solvents, oligomers, polymers, and interfaces which interact with structural organization can dramatically influence biomolecule function.[31−34]

(d) Proteins are dynamic: local and/or global conformational motions are central to ligand capture/binding and the resulting recognition, transduction, and catalytic event(s).[31,32] Dynamics can range from relatively small structural changes restricted to the region of the binding site as in proteases, to the movement of secondary structures as found in lipases and cutinases, and to large-scale concerted structural rearrangements in oligomeric assemblies as observed for photoactive and membrane proteins. Hence, any environmental factors (solvents, interfaces, etc.) that have an impact on the kinetics and/or thermodynamics of structural transitions can profoundly impact protein functioning.

Considering the above, one can identify several critical aspects that need to be borne in mind when considering the sol−gel bioencapsulation of proteins:[16−21,30,31,33,34]

(a) The encapsulation of biomolecules should proceed via their aqueous solutions, native hydrated forms, or some "protected" form which enables the recovery of native activity thereafter: (1) using aqueous solutions or suspensions of biomolecules, or stabilized forms, assemblies with synthetic surfactants, natural lipids, membranes, or protein arrays, or solid-supported nanosized dispersions;[29,30,34] (2) alternatively, biomolecules can first be stabilized by conjugation with surfactants, PEG, siloxanes, silica, and acrylates and then encapsulated using nonaqueous polymer chemistries.[30]

(b) Encapsulation and subsequent processing must be biocompatible: (1) aqueous protocols must employ media at ambient/subambient temperatures, in the biological pH and redox range, at low ionic strength, and with the minimal use of solvents or other organic species which can cause protein unfolding/denaturation, aggregation or precipitation, or other deleterious structural effects;[16−29] (2) nonaqueous media permit the use of more aggressive conditions by virtue of the greater stability of the biomolecule conjugates employed, and solvents, surfactants, and redox and free radical initiators can be used.[30]

(c) The formed polymer structure must allow for sufficient access to the protein as well as its requisite conformational mobility, while preventing its leakage from the matrix:[16−29] a continuous mesocellular framework with direct entry to the protein's binding site is essential for avoiding diffusional hindrances, as is a suitably unrestrictive entrapment cavity for permitting essential molecular motions. However, there must be sufficient constraint in the form of physical embedding, interface templating, and/or specific chemical interactions to preclude biomolecule leaching from the polymer matrix.

These considerations are more critical for multimeric proteins and proteins which are part of assemblies such as bilayers, vesicles, and membranes, as the gross structural integrity and large-scale internal mobilities of these structures must be preserved.[34−36] The most stringent requirements are encountered with organelles, and ultimately living cells, which require not only mild encapsulation conditions substantially devoid of toxic incidince but also nonintrusive encapsulants which must not disrupt physical unity or the delicate functioning of countless biochemical processes.[37,38]

The Essentials of Sol−Gel Nano-bioencapsulation

The field of bio-doped nanocomposites started with, and has centered on, sol−gel materials,[29] with the first publications establishing some critical features which form the basis of the present day technology:[16−20,29]

(a) Aqueous sol−gel protocols can be modified and used to encapsulate labile biomolecules to form biodoped nanocomposites.

(b) The judicious selection of experimental conditions enables bioencapsulation under sufficiently mild conditions that biomolecules can retain their native structure, dynamics, and molecular recognition and catalytic functions, in both hydrogels and xerogels.

(c) The mesoporous structure and high pore volume of sol−gels permit the diffusion of low- to medium-molecular-weight species and their free interaction with the recognition sites of entrapped biomolecules.

(d) The mesoporosity and framework rigidity of sol−gel polymers prevent the leakage of entrapped biomolecules, while also stabilizing their structure.

With these realizations has come the recognition that sol−gel technology offers some unique advantages for the immobilization of biological materials:[16−29]

(a) Sol−gel polymerization offers the only route to date for incorporating otherwise labile biomolecules into physicochemically robust ceramics to form true nanocomposites.

(b) The intrinsic silicon-chemistry-based flexibility of sol−gel polymers can be used to effect bioencapsulation in a diverse range of inorganic, hybrid organic−inorganic, and composite materials.

(c) Inorganic and some hybrid sol−gels can be fabricated as optically transparent glasses suitable for UV−vis use and hence applied as optical materials.

(d) Redox-active sol−gels can be synthesized using transition metal oxide sol−gels and/or incorporating conducting/redox components such as graphite, noble metals, metallocenes, organic dyes, and redox cofactors, thus allowing access to electrochemical devices.

(e) Conventional fabrication methods can be used to form bioencapsulates as monoliths, nano-, micro-, and macroparticulates, and passive-deposited, spun, screen-printed, and stamped thin and thick films.

The procedure for bioencapsulation parallels that of standard sol−gel entrapment, except that the protocol is modified to ensure that polymerization and processing are conducted at biocompatible pHs, redox conditions, and temperatures and that solvents and reactive/toxic organic species are minimized. It is usually implemented as follows (Figure 1):[5,16−29]

(1) An aqueous sol composed of partially or fully hydrolyzed alkoxysilanes is prepared:

(a) Alkoxysilanes are 50−100% hydrolyzed using acid- and/or surfactant-mediated catalysis in water or water−alcohol mixtures to furnish solutions of poly(alkoxysiloxanes), poly(alkyl silicates), or poly(silicic acids), which may be fully evaporated to remove alcohols.[5,16−28]

(b) Alkoxysilanes are 50−75% hydrolysis and transesterified with glycerol under acidic, basic, or alkoxide catalysis in alcohol solvents, to form water-soluble isolable poly(glyceryl silicates) and poly(glyceroxysiloxanes) which are dissolved in water.[29]

The constitution of the sol ranges from a mixture of linear, branched, and cyclic poly(silicates)/poly(siloxanes) with DPs of 3−16 for partially hydrolyzed sols to colloidal sols of nanometeric macromers for fully hydrolyzed and aged sols. The exact makeup depends on the structure of the precursor silane (number and types of alkoxy groups, functionality of nonhydrolyzable moieties, whether silane or disiloxane, etc.), its concentration, hydrolysis conditions, presence of solvents, and aging history of the sol.[5,16,19,20,29]

(2) This precursor sol is mixed with a buffered (pH 5−9) solution or suspension of the biological, which may also contain fluoride or amine catalysts, and drying

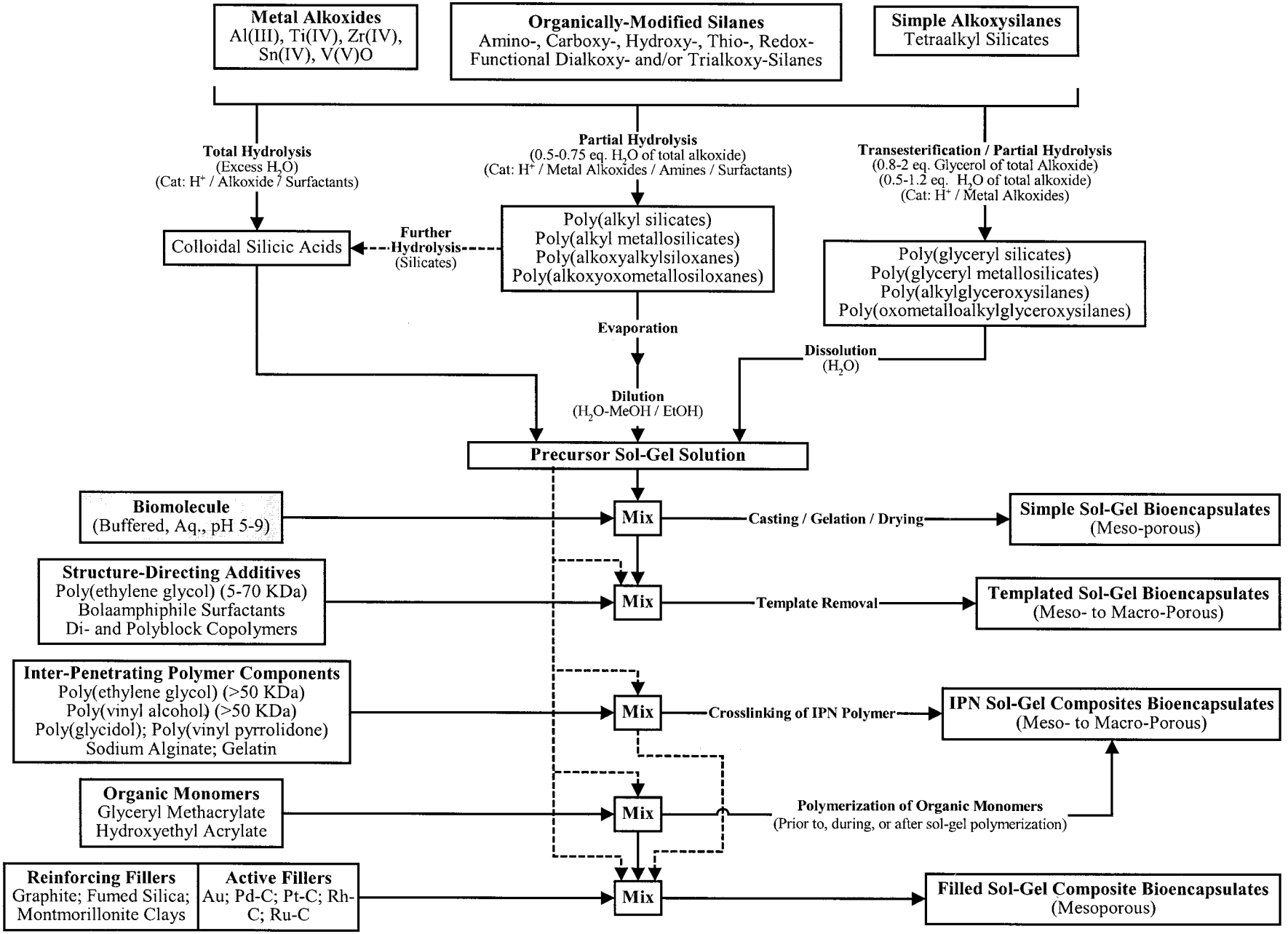

Figure 1. General protocols for sol−gel bioencapsulation.

control additives such as formamide and polyols. Structure modifiers such as PVA and PEG and reinforcing fillers such as graphite powder and fumed silica can also be included. The catalysts and/or elevated pH initiate condensation, cross-linking, and phase separation events which result in an increase in viscosity, and culminate in sol−gel transition and bulk gelation, with concomitant bioencapsulation:

(a) General acid/base catalysis of hydrolysis/condensation reactions by surface amine, amide, hydroxy, thiol, and carboxy residues of the protein and functional additives.

(b) Adsorption of silicate/siloxane oligomers onto biomolecule surfaces via ionic, H-bonding, and/or hydrophobic interactions to form protein−macromer complexes and the directing of oligomer growth and morphology via protein topology and surface chemistry. Additives such as PEG and PVA can similarly direct structural evolution.

(c) Partial embedding of the biomolecule via the overgrowth of protein−macromer complexes and/or their coalescence to form colloidal nanoparticles.

(d) The further growth, coalescence, and ensuing phase separation of the nanoparticles to form fused nano- or microparticle frameworks, coinciding with gelation and formation of the hydrated mesoporous framework.

It is important to minimize the use of polar solvents in this stage—at 0−30% v/v alcohols and ketones can lead to reversible aggregation and inhibition, while higher concentrations often cause irreversible denaturation and precipitation with corresponding losses in biological activity.[16−20,29] The formed hydrogels are typically brittle or semiflexible, compressible gels that contain 50−80% interstitial water, with pore volumes of 0.9−3.4 mL g^{-1}, pore distributions of 4−200 nm, and surface areas of 600−2100 m^2 g^{-1}. The extent of encapsulation depends on the nature of the biomolecule, the type of sol−gel precursor used, and the gelation conditions—as a rule, silicates and hydrophilic siloxanes give near quantitative immobilizations, while the entrapment efficiencies of hydrophobic siloxanes may fall below 50% due to phase separation effects. Because of the high degree of hydration and elevated porosity, biomolecules typically display 60−100% of their native activities within fresh hydrogels.[16−20,29]

(3) The hydrogel is aged in the wet state for 12−72 h to allow for the completion of condensation reactions, particle growth/fusion, and the maturation of the pore network. Cross-linking results in bulk shrinkages of 5−30% and therein contraction of the pore network and expulsion of entrained liquid via syneresis. Aged hydrogels range from brittle glassy silicate and hydrophobic siloxane gels to semiflexible hydrophilic siloxane and composite hydrogels, with typical pore volumes, are 0.4−3.1 mL g^{-1}, pore sizes 3−150 nm, and surface areas 600−1700 m^2 g^{-1}. Aging can reduce biomolecule activities by 0−20% from those observed in fresh hydrogels.[16−20,29]

(4) The aged hydrogel is washed with buffer and then dried, resulting in the loss of most of the remaining water, further cross-linking and structural consolidation, pore collapse, and bulk shrinkage. Controlled desiccation, via freeze-drying or pinhole drying, may be required to avoid extreme structural collapse and loss of porosity, which can result in a severe loss of biomolecule viability. The incorporation of drying control additives or the use of glyceroxysilane precursors can substantially reduce pore collapse effects, speed up the drying process, and enable the recovery of high levels of biomolecule activity.[29] Xerogels range from optically clear brittle silicate glasses to rigid or semiflexible siloxane plastics and rubbers, which contain up to 30% of bound water, show pore volumes of 0.3−2.7 mL g^{-1}, pore sizes of 0.5−100 nm, and surface areas of 400−1300 m^2 g^{-1}. Because of their desiccated state and resulting pore collapse and matrix compression, up to 80% of biomolecule viability can be lost in the final xerogel. Rehydration of xerogels can result in a considerable expansion of the pore framework and the recovery of up to 30% of biomolecule activity, especially in the case of xerogels derived from hydroxylated siloxanes and composite xerogels PVA, PEG, alginate, gelatin, poly(hydroxyethyl acrylate), and so forth.

Types of Sol−Gel Precursors and Matrixes

Initial efforts in sol−gel bioencapsulation largely focused on silica matrixes derived from colloidal silicic acid or tetraalkyl silicates[10−15] because of their ready synthesis, physicochemical robustness, and optical transparency. A wide range of matrixes have since augmented silica encapsulants (Table 1, Figure 1):[1−4,16−29,39]

Inorganic sol−gels: Aluminum, titanium, zirconium, tin, vanadium, and molybdenum oxides, their mixed oxides with silica, and polyoxomolybdate-silicas. The xerogels are hard transparent glasses that are micro- to mesoporous, are chemically robust, and have good optical clarity, but are limited by their brittleness, limited porosity, and lack of modifiable chemical functionality.

Organically modified silica sol−gels (Ormosils): The precursor silanes bear organic groups attached by hydrolytically stable Si−C bonds and furnish poly(organosiloxanes) with an inorganic siloxane backbone with pendant organic moieties. Attached functions range from simple alkyl, alkenyl, and aryl to those additionally bearing amino, amido, carboxy, hydroxy, thiol, and mixed functionalities as well as redox-active metallocenes, nicotinamides, flavins, and quiniones. The matrixes offer tailorable hydrophilic, hydrophobic, ionic, and H-bonding capacities as well as electrochemical activities and display good porosities. However, the hydrogels and xerogels are relatively fragile, have limited optical transparency, and are less robust than the inorganic matrixes.

Hybrid sol−gels: These comprise amino- and/or hydroxy-functional functional homopolymers and diblock copolymers, such as polymethylsiloxane, poly(dimethylsiloxane), poly(alkene oxide), polyurethane/polyurea, polyacrylate, polyphosphazene, and poly(dimethylsiloxane)-*co*-poly(alkene oxide), which are main-chain or periphery modified with alkoxysilanes.[29,39] Polymerization provides siloxane-cross-linked dendritic or comb architectures, combining the physicochemical attributes of the component polymers. Although rigid, semirigid, rubber, and plastic materials with good mechanical properties, variable hydrophobic−hydrophilic balances, and porosities are accessible, they have poor optical

Table 1. Types of Sol−Gel Polymers Utilized for Bioencapsulation and Their Typical Precursors

Sol-Gel Polymer	Typical Sol-Gel Precursors	Sol-Gel Bulk Properties	Sol-Gel Chemical Properties
Silica, Metal Oxides and Metallosilicates			
Silicas	Colloidal silicas/silicic acids Tetraalkyl silicates: $Si(OMe)_4$; $Si(OEt)_4$; $Si(OiPr)_4$ Poly(alkyl silicates): $[SiO_{1-1.4}(OMe)_{1.2-2}]_n$ Poly(glyceryl silicates): $[SiO_{1-1.2}Glc_{0.8-1}]_n$	Hard - Brittle Clear - Transparent UV-VIS	Hydrophilic - Semi-hydrophobic Stable to pHs 2-9 Degraded by fluorides
Aluminium Oxide (Aluminosilicates)	Aluminium(III) alkoxides: $Al_2(OEt)_6$; $Al_2(OiPr)_6$; $Al_2(OtBu)_6$ Aluminium(III) alkoxides + poly(alkyl silicates), poly(glyceryl silicates), etc	Hard - Brittle Clear - Transparent UV-VIS	Hydrophilic Stable to pHs 2-14
Zirconium Oxide (Zirconosilicates)	Zirconium(IV) alkoxides: $Zr(OEt)_4$; $Zr(OiPr)_4$; $Zr(OtBu)_4$ Zirconium(IV) alkoxides + poly(alkyl silicates), poly(glyceryl silicates), etc	Hard - Brittle Clear - Transparent UV-VIS	Hydrophilic Resistant to pHs 2-14
Titanium Oxide (Titanosilicates)	Titanium(IV) alkoxides: $Ti(OEt)_4$; $Ti(OiPr)_4$; $Ti(OtBu)_4$ Titanium(IV) alkoxides + poly(alkyl silicates), poly(glyceryl silicates), etc	Hard – Brittle Clear - Transparent UV-VIS	Hydrophilic Stable to pHs 2-14
Tin(IV) Oxide	Tin(IV) alkoxides: $Sn(OEt)_4$; $Sn(OiPr)_4$; $Sn(OtBu)_4$ Tin(IV) alkoxides + poly(alkyl silicates), poly(glyceryl silicates), etc	Hard - Brittle Clear -Semi-transparent UV-VIS	Hydrophilic Conductive - Redox-active Stable to pHs 4-10
Vanadium(V) Oxide	Vanadium(V)oxide trialkoxides: $VO(OEt)_3$; $VO(OiPr)_5$ Vanadium(V)oxide trialkoxides + poly(alkyl silicates), poly(glyceryl silicates), etc	Hard - Brittle Clear - Coloured - Opaque UV-VIS	Hydrophilic Conductive - Redox-active Photochromic Stable to pHs 3-9
Molybdenium(V) Oxide	Molybdenum(V) alkoxides: $Mo(OEt)_5$; $Mo(OiPr)_5$; $Mo(OtBu)_5$	Hard - Brittle Clear - Coloured - Opaque UV-VIS	Hydrophilic Conductive - Redox-active Stable to pHs 4-10
Polyoxometallates-Silicates-Siloxanes	Silicomolybdic acid, phosphomolybdic acid, phosphotungstomolybdic acid + tetraalkyl silicates, poly(alkyl silicates), poly(glyceryl silicates), etc	Hard - Brittle Clear - Coloured Opaque UV-VIS	Hydrophilic - Semi-hydrophobic Redox-active Stable to pHs 3-9
Organically-Modified Siloxanes (ORMOSILs)			
Alkylsiloxanes	Trialkoxyalkylsilanes: $SiR'(OR)_3$ Dialkoxydialkylsilanes: $Si(R')_2(OR)_2$ Tetraalkoxydialkyldisiloxanes: $[OSiR'(OR)_2]_2$ Bis(trialkoxysilyl)alkanes: $(C_nH_{2n})[Si(OR)_3]_2$ (R′ = CH_3 to $C_{18}H_{37}$, cyclohexyl; R = Me, Et, Pr, glyceryl)	Soft - Semi-flexible - Hard – Brittle Clear - Translucent - Opaque Semi-transparent UV-VIS	Hydrophobic Stable to pHs 2-10
Alkenylsiloxanes Alkylalkenylsiloxanes Alkynylsiloxanes	Trialkoxyalkenylsilanes: $SiR'(OR)_3$ Dialkoxyalkylalkenylsilanes: $SiR'R''(OR)_2$ Tetraalkoxydialkenyldisiloxanes: $[OSiR'(OR)_2]_2$ *N*-Acryloylaminoalkyltrialkoxysilanes: $CH_2CHCONHC_3H_6Si(OR)_3$ (R′ = vinyl, allyl, propargyl; R″ = Me, Et; R = Me, Et, glyceryl)	Soft - Semi-flexible - Hard – Brittle Clear - Translucent - Opaque Semi-transparent UV-VIS	Semi-hydrophobic Stable to pHs 2-10
Arylsiloxanes Arylalkylsiloxanes	Trialkoxyarylsilanes: $SiAr(OR)_3$ Bis(trialkoxysilyl)arenes: $Ar[Si(OR)_3]_2$ (Ar = Ph, $PhCH_2$, C_6H_4, $C_6H_4(CH_2)_2$; R = Me, Et)	Soft - Semi-flexible - Hard - Brittle Clear - Translucent - Opaque Semi-opaque UV-VIS	Hydrophobic Stable to pHs 2-11
Aminosiloxanes	Aminoalkyltrialkoxysilanes: $NH_2C_3H_6Si(OR)_3$ *N,N*-Bis(aminoalkyl)aminoalkyltrialkoxysilanes: $(NH_2C_2H_4)_2NC_3H_6Si(OR)_3$ *N*-(Aminoacyl)aminoalkyltrialkoxysilanes: $AAc\text{-}NHC_3H_6Si(OR)_3$ (Aac = 2-aminoacyl; R = Me, Et, Pr, glyceryl)	Semi-flexible Clear - Semi-transparent UV-VIS	Hydrophilic Basic - Zwitterionic Stable to pHs 6-11
Amidoalkylsiloxanes	*N*-acylaminoalkyltrialkoxysilanes: $R'CONHC_3H_6Si(OR)_3$ Ureidoalkyltrialkoxysilanes: $NH_2CONHC_3H_6Si(OR)_3$ (R′CO = Ac, TFAc, higher acyl; R = Me, Et, Pr, glyceryl)	Semi-flexible - Brittle Hydrogels Clear - Semi-transparent UV-VIS	Hydrophilic - Semi-hydrophobic Basic - Neutral Stable to pHs 4-11
Carboxyalkylsiloxanes	Carboxyalkyltrialkoxysilanes: $HO_2CC_3H_6Si(OR)_3$ *N*-(Carboxyalkylacyl)aminoalkyltrialkoxysilanes: $HO_2CCH_2CONHC_3H_6Si(OR)_3$ (R = Me, Et, Pr, glyceryl)	Semi-flexible - Brittle Hydrogels Clear - Semi-transparent UV-VIS	Hydrophilic Acidic Stable to pHs 2-11
Hydroxyalkylsiloxanes	Dihydroxyalkyltrialkoxyalkylsilanes: $HOCH_2(HOCH)OC_3H_6Si(OR)_3$ *N,N*-Bis(hydroxyalkyl)aminoalkyl: $(HOC_2H_4)NHC_3H_6Si(OR)_3$ *N*-(Polyhydroxyalkyl)aminoalkyltrialkoxysilane: $R'CONHC_3H_6Si(OR)_3$ Hydroxyalkylureidoalkyltrialkoxysilane: $HOC_2H_4NHCONHC_3H_6Si(OR)_3$ (R′CO = δ-gluconyl, pantoyl; R = Me, Et, Pr, glyceryl)	Flexible - Semi-flexible - Rigid Hydrogels Clear - Semi-transparent UV-VIS	Hydrophilic Neutral - Basic Stable to pHs 4-11
Mercaptoalkylsiloxanes Sulphidoalkylsiloxanes	Thioalkyltrialkoxyalkylsilanes: $HSC_3H_6Si(OR)_3$ Bis(trialkoxysilylalkyl)disuphides: $[SC_3H_6Si(OR)_3]_2$ (R = Me, Et, Pr, glyceryl)	Flexible - Semi-flexible - Rigid Hydrogels Clear - Semi-transparent UV-VIS	Hydrophilic Neutral - Slightly acidic Redox-active Stable to pHs 4-9
Redox-active Siloxanes	Ferrocenylalkyltrialkoxysilanes: *N*-Ferroceneacetyl-$NHC_3H_6Si(OR)_3$ Nicotinamidoalkyltrialkoxysilanes: [NAD-/NADP-CONH]-$COC_3H_6Si(OR)_3$ Flavinamidoalkyltrialkoxysilanes: [FAD-NH]-$COC_3H_6Si(OR)_3$ (R = Me, Et, Pr)	Flexible - Semi-flexible - Rigid Clear - Coloured Semi-transparent UV-VIS	Hydrophilic Redox-active Stable to pHs 5-9
Hybrid Sol-Gel Materials			
Polydimethylsiloxanes-Silicates-Siloxanes	Bis(alkoxyalkylsiloxy)polydimethylsiloxane: $[SiR'(OR)_2O]_2[Me_2SiO]_n$ Bis[poly(alkoxyalkylsiloxy)]polydimethylsiloxane: $\{[(SiO_xR'(OR)_y]_m\}_2[Me_2SiO]_n$ (R′ = aminoalkyl, hydroxyalkyl, etc; R = Me, Et, Pr, glyceryl)	Flexible - Semi-flexible - Rigid Translucent - Opaque Semi-transparent UV-VIS	Hydrophobic Stable to pHs 4-11
Polymethylsiloxane-Silicates-Siloxanes	Poly[(trialkoxysilylethylene)methylsiloxane]: $\{[(OR)_3SiC_2H_4]Si(Me)O\}_n$ (R = Me, Et, Pr, glyceryl)	Flexible - Semi-flexible - Rigid Translucent - Opaque Semi-transparent UV-VIS	Hydrophobic - Hydrophilic Surface-active Stable to pHs 4-11

Table 1 (Continued)

Sol-Gel Polymer	Typical Sol-Gel Precursors	Sol-Gel Bulk Properties	Sol-Gel Chemical Properties
Poly(alkene oxides)-Silicates-Siloxanes	Bis(alkoxyalkylsiloxy)poly(ethylene oxide): $[Si(OR)_2C_3H_6NHCH_2(CHOH)]_2$-PEO Bis(alkoxyalkylsiloxy)-(PEO-*co*-PDMS): $[Si(OR)_2C_3H_6NHCH_2(CHOH)]_2$-(PEO-*co*-PDMS) (R = Me, Et, Pr, glyceryl)	Flexible - Semi-flexible - Rigid Translucent - Opaque Semi-transparent UV-VIS	Hydrophobic - Hydrophilic Surface-active Stable to pHs 4-10
Poly(acrylates)-Silicates-Siloxanes	Poly[(trialkoxysilylalkylaminocarboxy)ethylmethacrylate): $[Si(OR)_3C_3H_6NHCO_2C_2H_4O_2C(Me)CH]_n$ (R = Me, Et, Pr, glyceryl)	Flexible Hydrogels Clear - Semi-transparent UV-VIS	Hydrophilic Stable to pHs 4-10
Polyurethanes-Silicates-Siloxanes Polyureas-Silicates-Siloxanes	Bis(trialkoxysilylalky)-[poly(propylene glycol)-diurea]: $[Si(OR)_3C_3H_6NHCONH(MeC_6H_3)NHCO(CH(Me)CH_2)_n]_2$ Tris[(trialkoxysilylalkyureido)poly(propylene glycol)]glycerol: $[CHO\text{-}(CH_2O)_2\text{-}]\text{-}[(CH_2CH(Me)O)_nCONHC_3H_6Si(OR)_3]_3$ (R = Me, Et, Pr, glyceryl)	Flexible Hydrogels Clear – Translucent Semi-opaque UV-VIS	Hydrophilic Stable to pHs 4-10
Poly(phosphazenes)-Silicates-Siloxanes	Poly[bis(trialkoxysilylalkylamino)phosphazenes]: $[NP(NHC_3H_6Si(OR)_3)_2]_n$ (R = Me, Et, Pr, glyceryl)	Flexible Clear - Translucent Semi-transparent UV-VIS	Hydrophilic Stable to pHs 4-10

properties, and in some cases are only available as hydrogels due to undue structural collapse upon drying.

Interpenetrating polymer network (IPN) composite sol−gels: These are nano- or microcomposite polymers prepared by the following:[29,39] (1) the combining of a sol−gel solution with a solution of a water-soluble polymer such as alginate, carrageenan, gelatin, agar, poly(vinyl alcohol), poly(ethylene glycol), poly(vinylpyrrolidone), or poly(glycidol), followed by sol−gel polymerization, and then organic polymer salt-cross-linking (alginate, carageenan) or cryo-phase separation (PVA, gelatin, agar, and gelatin); (2) nonhydrolyzed or partially hydrolyzed silicate/siloxane precursors infused into organic hydrogels such as calcium alginate, potassium carageenan, agar, gelatin, PVA cryogels, poly(glyceryl methacrylate), poly(hydroxyethyl acrylate), poly(acrylamide), polyurethane, and so forth and sol−gel polymerization allowed to proceed therein; (3) organic monomers such as glyceryl and hydroxyethyl acrylates infiltrated into preformed sol−gels and polymerized via free radical, UV, or redox initiation. Composites are produced which typically contain 20−80% w/w of organic component, display varying degrees of phase separation, and form rigid plastics, elastic hydrogels, and flexible rubbers with meso- to macropore networks. The presence of the sol−gel phase greatly alters the properties of the organic polymer—soft hydrogels which readily imbibe water and/or are liquefied by chelating agents or elevated temperatures are transformed into rigid glassy materials which are resistant to swelling and structural collapse, because of the supporting action of the supporting sol−gel framework. The composites are often highly biocompatible and are highly suited to the entrapment of very labile biologicals such as membrane fragments, organelles, and living cells.

Reinforced/filled composite sol−gels: The above materials can be reinforced with inert nano- or microparticulate materials such as native and oxidized graphite powder, fumed silica, methylated-silica, clays, cellulose, and so forth.[1−3,16−29,39] These materials can be incorporated at levels of up to ca. 75% w/w and act as reinforcing fillers to improve the mechanical properties and processing behavior of the sol−gels. In addition, active fillers such as gold, palladium, platinum, and palladium-graphite can be used where conducting and redox-active materials are desired.

Templated sol−gels: Simple sol−gel systems can be templated with structure-directing and pore-forming agents, including polyols, hydroxyacids, PEG, monovalent, gemini, and bola-amphiphile surfactants and surface-active di- and polyblock copolymers.[1−4,40−52] These compounds form microemulsions, vesicular or liquid-crystalline phases, macroemulsions, foams, or H-bonded aggregates, and therein direct sol−gel formation, or phase-separate during mixing/sol gelation and thereafter modify sol−gel structure. Removal of the templates after aging and drying provides meso- to macroporous materials with ordered pore frameworks. Although scant little has been done on applying such additives to bioencapsulation, the availability of biocompatible templating agents holds great promise for accessing highly ordered and porous bio-doped sol−gels.[39,50]

In general, the physicochemical properties of hybrid and composite materials are enhanced over those of the constituent sol−gel polymers due to the reinforcing action of the particulate or interpenetrating phase.[1−3,29,39] Similarly, xerogels are superior in mechanical properties and chemical resistance to hydrogels by virtue of cross-linking and densification.[16−20] On the other hand, the drying of hydrogels inevitably reduces porosity, increases steric compression and diffusional limitations, and results in a reduced bioactivity, especially for inorganic sol−gels.[16−29] Also, most membrane assemblies and most organelles and cells cannot survive complete dehydration, and are very sensitive to structural disturbances caused by invasive polymer matrixes, and for these hydrophilic sol−gel hydrogels are often essential for preserving viability.[17,29,39]

Structure of Bio-doped Sol−Gels and Biological Functioning Therein

While the gross morphology of bio-doped sol−gels is similar to that of undoped materials, the presence of biomolecules may substantially affect sol−gel microstructure by modifying its initiation, development, and consolidation. Thus, acidic/basic and charged residues influence the mechanism and rates of alkoxysilane hydrolysis and condensation, and charged, hydrophilic, hydrophobic, and H-bonding domains can act as interfacial templates to direct the branching pattern, functionality, growth, topology, and aggregation of developing polysilicate/polysiloxane colloids.[1−3,20−29,40−48] Such biomolecule-directed structure evolution has been postulated to explain differences in the microstructure of bio-doped sol−gels, although detailed spectroscopic and

microscopic studies to elucidate the role of such processes have as yet to be presented.

The mode of entrapment of large oligomeric proteins and micron-sized biologicals such as membrane fragments, organelles, and cells that are larger than the pore structure is readily apparent. However, the mechanism of encapsulation and retention of smaller biomolecules whose dimensions are smaller than the pore size of the sol−gel is more intriguing. Thus, proteins with molecular weights as low as approximately 8000 (ca. 1 nm) can be irreversibly encapsulated in sol−gels whose mesopore networks span 2−6 nm and allow the penetration of molecules with masses of 2000−200 000. Thus, polylysine with a molecular weight of over 150 000 can penetrate silica and metallosilicate xerogels and therein inhibit trypsin,[39,53] and cytochrome *c* and myoglobin are able to diffuse through bola-siloxane xerogels.[54] Similarly, RNAse enzymes can infiltrate silica xerogels and bind to encapsulated DNP-poly(adenylate),[55] and immunoglobulin antibodies (IgGs) can diffuse into silica hydrogels and bind encapsulated antigenic proteins and cells.[56] The most dramatic results are seen with high-porosity sol−gels, which can be prepared via special gelation and templating procedures with pore sizes of 2−20 nm (xerogels) and 4−100 nm (hydrogels).[16−29,39−52,57,58] Thus, proteins with molecular weights of 14 000−77 000 can be efficiently fractionated by triblock copolymer-templated mesocellular silica xerogels.[57] In a most intriguing demonstration of the porosity and pore connectivity of sol−gels, it has been shown that intact 50−100-nm influenza-virus particles can access and bind to sialic-acid-coated liposomes encapsulated in silica hydrogels.[58]

Investigations to date suggest that bioentrapment derives from physical embedding within sol−gel nanoparticles and/or strong binding interactions between protein and biomolecule-templated sol−gel surfaces sufficient to preclude subsequent leaching. Indeed, studies indicate that small- to mid-sized biomolecules (5000−100 000 MW) are fully or partially encased by sol−gel surfaces that substantially conform to the hydrated topology of the biologicals, thereby preventing their diffusion.[16−29] While global biomolecule dynamics are restricted, it appears that the encapsulating sol−gel structure of all except the most consolidated xerogels is sufficiently loose to allow for local rotational and translational transitions.[16−18]

Spectroscopic investigations of myoglobin, hemoglobin, cytochrome *c*, cytochrome *c* peroxidase, catalase, monellin, oncomodulin, glutamate dehydrogenase, glucose oxidase, and nitrobenzoxadiazole- and acryloidan-labeled bovine and human albumins entrapped in silica and siloxane hydrogels and xerogels indicate that the proteins are sequestered within 3−10-nm hydrated pockets wherein they substantially retain nanosecond and sub-nano-second dynamics and at least aspects of global mobility, akin to the dissolved biomolecules.[59−79] Indeed, fluorescence studies of silica-entrapped glucose oxidase indicate that the rotational mobility of the enzyme is approximately 50% of that in solution, that the folding kinetics of the flavin moiety are greatly reduced, but that the local motions of the active site pocket are similar to those in solution.[69,78] Similarly, the silica-entrapped photoactive proton-pump bacteriorhodopsin shows a decrease in the rate of decay of its excited M state, but retains enough global mobility to change its conformational state and therefore function.[80−84]

As well as physically restricting biomolecule motions, the polymer surface can physically block the recognition/catalytic site. For instance, studies with myoglobin, albumins, and glucose oxidase have demonstrated that the active sites of subpopulations of the silica-entrapped proteins are in highly restricted environments which are nonaccessible to ligands.[60−63,78,81,82] Likewise, the chemical functionality of the enveloping sol−gel surface can strongly affect protein function.[16−29] Thus, glucose oxidase retains most of its activity, while glycolate and lactate oxidases lose most of their functioning, when encapsulated in silica.[85,86] It has been postulated that structures of the active sites and flavin function in the latter enzymes is disrupted via electrostatic interactions between their positively charged binding channels and the anionic silica matrix, while the zwitterionic nature of the binding cleft in glucose oxidase prevents such deleterious effects.

From the above, one can envisage the following structure for bio-doped sol−gels:[16−29]

(a) The biological is embedded within the body of the polymer matrix, or within the percolating pore structure of, a bicontinuous framework of fused nano- or microparticles which enclose a disordered or highly structured mono- or polymodal or hierarchical micro-, meso-, and/or macropore network.

(b) Depending upon the biological, and the composition and mode of preparation of the sol−gel, the biomaterial may be distributed homogeneously as isolated molecules, may self-associate/precipitate during gelation to form aggregates, or may be nano- or microcompartmentalized at the surface of sol−gel particles or into the pore structure due to interfacial/phase separation effects.

(c) The biological is in contact with a partially or fully enclosing polymer shell, the contact surface of which is templated to varying degrees to conform topologically and chemically to the hydrated surface of the biomolecule.

(d) A high-viscosity H-bonded layer of water is trapped between the biomolecule and polymer surfaces.

(e) Global biomolecule mobility and segmental motions are diminished depending upon the degree of compliance of the biomolecule and polymer surfaces, the type and magnitude of their interactions, and the amount and mobility of the water layer.

(f) There is sufficient accessibility between the recognition sites of a portion of the biomolecule population and the enveloping pore structure and enough freedom for local conformational transitions to enable the entry and recognition/reaction of ligands.

Applications of Sol−Gel Bioencapsulates: Biosensors to Biocatalysts

The most investigated area of application of sol−gel bioencapsulates has been the realm of biosensors.[16−29] There is a pressing demand for rugged, miniaturized, and portable biosensing devices across fields as diverse as in vivo medical monitoring, diagnostics, bioprocess monitoring, food analysis, environmental monitoring,

Table 2. Examples of Glucose Oxidase (GOx) and GOx−Horseradish Peroxidase (HRP) Sol−Gel Optical and Electrochemical Biosensors for Glucose[a]

Precursors	Sensor Configuration	Sensor Type	Ref
TEOS	[GOx ± HRP + 1,1'-dimethylferrocene]-carbon-silica xerogels (Screen-printed thick film electrodes on alumina)	Amperometric	100
MeTMOS	[GOx ± HRP]-[Pd-graphite]-siloxane or [GOx-ferrocene conjugate]-graphite-siloxane xerogels (Monolithic electrodes)	Amperometric	103, 111
TEOS	[GOx]-carbon-silica xerogels (Screen-printed thick film electrodes on alumina)	Amperometric	104
MeTMOS TEOS	[GOx]-Au-siloxane xerogels (Screen printed thick film electrodes on alumina)	Amperometric	105
3-FAPTMOS MeTMOS	[GOx]-carbon-siloxane xerogels (Monolithic electrodes)	Amperometric	107
TEOS	[GOx]-silica xerogels (Thin films dip-coated on Pt microelectrodes)	Amperometric	109
TMOS	[GOx]-silica xerogels (Thin films on poly(1,2-diaminobenzene)-coated Pt electrodes)	Amperometric	110
TEOS 3-FAPTMOS	[GOx + HRP]-graphite-siloxane xerogels (Monolithic electrodes)	Amperometric	113
TEOS	[GOx]-silica xerogels (Particulate in flow cell, sandwiched with O_2-sensitive [$Ru(dpp)_3(DS)_2$-doped silica xerogel]-silicone composite optode membrane	Fluorescence	114
MeTMOS TMOS	[GOx]-siloxane xerogel (Thin film on thin film O_2-sensitive [$Ru(dpp)_2$]-siloxane xerogel optode, on silica) ([GOx]-siloxane thick film with [$Ru(dpp)_2$]-siloxane xerogel particles, on silica)	Fluorescence	115
TMOS	[GOx-Fluorescein/Texas Red/Cy5 bioconjugates]-silica hydrogels (Thick films on polystyrene or silica)	Fluorescence	116
3-APTEOS 2-ECETMOS	[GOx]-siloxane hydrogels crosslinked with glyoxal (Thick films on Pt electrodes)	Amperometric	117
ATIP	[GOx]-alumina xerogels (Thin films on platinized glassy carbon electrodes)	Amperometric	118
MeTMOS	[GOx + vinylferrocene]-carbon-siloxane xerogels (Monolithic electrodes)	Amperometric	102, 103, 119
MeTEOS	[GOx]-[poly(vinylpyrrolidone)-$Os(bpy)_2$]-siloxane xerogels (Thin films on glassy carbon electrodes)	Amperometric	121
TMOS	[GOx + Co(II) phthalocyanine + ferrocene + DPTC]-carbon-silica xerogels (Screen-printed thick film electrodes on PVC or alumina)	Amperometric	122
TMOS	[GOx]-silica xerogels (Thin film on [ferrocene]-silica xerogel thin film on carbon paste electrode)	Amperometric	123
3-APTMOS MeTEOS TEOS	[GOx]-silica-siloxane hydrogels (Thick films between cellulose acetate membranes, on Pt H_2O_2 sensor) (Thin films on Pt microelectrodes, coated with [ferrocene]-silica xerogel thin film)	Amperometric	124
MeTMOS	[GOx]-[Pd-carbon]-siloxane xerogels (Monolithic electrodes)	Amperometric	125
TEOS	[GOx]-[poly(vinyl alcohol)-*g*-poly(4-vinylpyridine)]-silica xerogels (Thin films on glassy carbon electrodes)	Amperometric	126
3-APTEOS 2-ECETMOS	[GOx ± HRP]-graphite-PEG-siloxane or [GOx]-PEG-siloxane hydrogels (Thick films on Pt electrodes)	Amperometric	112, 127
MeTMOS	[GOx]-[Rh-graphite]-silica xerogel (Monolithic electrodes and screen-printed thick film electrodes on alumina)	Amperometric	128
MeTMOS	[GOx]-silica xerogel (Thin film on SAM-modified gold-coated graphite electrode)	Amperometric	129
TEOS	[GOx]-[Ru-graphite]-silica xerogels (Screen-printed thick film electrodes on alumina)	Amperometric	130
MeTMOS 3-FAPTMOS	[GOx]-graphite-siloxane xerogels (Monolithic electrodes)	Amperometric	103, 131
MeTMOS 3-APTMOS 3-FAPTMOS	[GOx]-graphite-siloxane xerogels (Monolithic electrodes)	Amperometric	132, 133

[a] Abbreviations: 3-APTMOS, 3-aminopropyltrimethoxysilane; 2-ECETMOS, 2-(3,4-epoxycyclohexyl)ethyltrimethoxysilane; 3-FAPTMOS, 3-ferroceneacetamidopropyl-trimethoxysilane; MeTEOS, methyltriethoxysilane; MeTMOS, methyltrimethoxysilane; TEOS, tetraethoxysilane; TMOS, tetramethoxysilane.

food and drug quality control, drug discovery, genomics, and proteomics.[87−98] The attraction of the sol−gel biosensing platform derives from its broad applicability to the encapsulation and stabilization of all classes of

Table 3. Examples of Other Oxidoreductase Sol−Gel Optical and Electrochemical Biosensors[a]

Enzyme(s)	Precursors	Sensor Configuration	Analyte(s)	Sensor Type	Ref
Peroxidase	TEOS	[Enz]-silica hydrogel (Thick film on polymethacrylate or silica optical fibres, coupled reaction with Luminol)	H_2O_2	Luminescence	134, 139
	TMOS	[Enz + ferrocene]-silica hydrogel (Thin film sandwiched between [ferrocene]-silica thin films, on carbon paste electrode)	H_2O_2	Amperometric	135
	TEOS	[Enz]-[poly(vinyl alcohol)-*g*-poly(4-vinylpyridine)]-silica hydrogels (Thick film on Nafion-Methylene Green-coated glassy carbon electrode)	H_2O_2	Amperometric	136
	TEOS	[Enz]-[poly(vinyl alcohol)-*g*-poly(4-vinylpyridine)]-silica hydrogels (Thick film on glassy carbon electrode, with $[Fe(CN)_6]^{4-}$ mediator)	H_2O_2	Amperometric	137
	MeTMOS 3-MPTMOS	[Enz]-[poly(vinyl alcohol)-*g*-poly(4-vinylpyridine)]-siloxane-Meldola's Blue hydrogels (Thick film on glassy carbon electrode)	H_2O_2	Amperometric	138
	TMOS	[Enz]-silica hydrogels (Thin film coated with silica hydrogel, on carbon paste electrode, with ferrocene mediator) (Determinations in MeOH, MeCN and *i*-PrOH solvents containing 20% v/v buffer)	H_2O_2 RO_2H RO_2R'	Amperometric	140
	TMOS	[Enz]-silica hydrogels (Thin film coated with silica hydrogel, on carbon paste electrode, with $[Fe(CN)_6]^{4-}$ mediator)	H_2O_2	Amperometric	141
	TEOS	[Enz]-[poly(vinyl alcohol)-*g*-poly(4-vinylpyridine)]-silica hydrogels (Thick film on glassy carbon electrode, with Methylene Blue mediator)	H_2O_2	Amperometric	142
	MeTEOS	[Enz]-[poly(vinylpyrrolidone)-Os(bpy)$_2$]-siloxane xerogels (Thin films on glassy carbon electrodes)	H_2O_2 CN^-	Amperometric	143, 144
	TEOS	[Enz]-[Pd-graphite]-silica xerogels (Screen printed thick films)	H_2O_2	Amperometric	145
Glucose, Galactose, Lactate Oxidases + Peroxidase	PGS 3-HEAGS	[Enzymes]-poly(vinyl alcohol)-siloxane hydrogels (Thick films)	Glc, Gal, Lactose, Lactate	UV-VIS	146
Lactate Oxidase	MeTMOS	[Enz] -[Pd-carbon]-siloxane xerogels (Monolithic electrodes)	Lactate	Amperometric	103, 125
	MeTEOS	[Enz]-[poly(vinylpyrrolidone)-Os(bpy)$_2$]-siloxane xerogels (Thin film on glassy carbon electrode)	Lactate	Amperometric	147
Oxalate Oxidase	TMOS	[Enz]-silica xerogels (Thick films and monoliths)	Oxalate	UV-VIS	148
Xanthine Oxidase	MeTMOS	[Enz]-graphite-siloxane xerogel (monolithic electrode, with $[Fe(CN)_6]^{4-}$ mediator)	Hypoxanthine	Amperometric	149
Polyphenol Oxidase	TEOS MeTMOS	[Enz]- [poly(vinyl alcohol)-*g*-poly(4-vinylpyridine)]-siloxane hydrogels (Thick film on glassy carbon electrode, response to catechol determined)	MeCN, *i*-PrOH, *n*-BuOH	Amperometric	150, 155
	TEOS MeTMOS 3-APTMOS	[Enz]-poly(ethyleneglycol)-siloxane or [PPOx]-poly(vinyl alcohol)-siloxane or [PPOx]-[poly(vinyl alcohol)-*g*-poly(4-vinylpyridine)]-siloxane hydrogels (Thick film on glassy carbon electrode, assays in pure buffer or buffer-saturated chloroform)	Phenol, Catechol, *p*-Cresol	Amperometric	151
	AlTIP	[Enz]-alumina xerogel (Thin film on $[[Fe(CN_6)]^{4-}]$-alumina xerogel thin film on glassy carbon electrode	Phenol	Amperometric	152
	TMOS	[Enz]-silica hydrogels (Thin film on glassy carbon electrode or thick films on [Co(II) phthalocyanine / Cu(II) phthalocyanine / ferrocene]-silica on carbon paste electrodes	Phenol, Catechol, 2-Chlorophenol, Cresols	Amperometric	153, 156
	ATIP	[Enz]-alumina xerogels (Thin films on glassy carbon electrodes)	Phenol, Amino-, Chloro-& Nitro-phenols, Catechol Cresols	Amperometric	154
Phenol Hydroxylase	TMOS	[Enz]-silica hydrogels (Thick films on Clark oxygen electrode)	Phenol, Amino-, Chloro- Fluoro-, Carboxy- & Methyl-phenols, Catechol, Resorcinol	Amperometric	157
Laccase	TMOS	[Enz]-silica hydrogels	Phenol, Catechol, Resorcinol, Aniline	UV-VIS	158
Cytochrome $P450_{cam}$	MeTEOS	[Enz + bovine serum albumin + didoceyldimethylammonium bromide liquid crystal]-glutaraldehyde-siloxane xerogels (Thin films on glassy carbon electrodes, assays in pure buffer or acetonitrile-buffer)	Camphor Pyrene	Amperometric	159
Choline Oxidase + Acetylcholine Esterase	PGS 3-GAPS	[Enzymes]-poly(vinyl alcohol)-[Pd-graphite]-siloxane hydrogels (Screen printed thick film electrodes)	Acetylcholine Choline	Amperometric	146
Lactate	TEOS	[Enz]-silica xerogels	Lactate, Pyruvate	UV-VIS	160
Dehydrogenase		(Thin films)			
Glucose-6-phosphate Dehydrogenase	PGS 3-APGS	[Enz]-poly(vinyl alcohol)-siloxane hydrogel (Monoliths, assay in presence of NADP)	Glucose-6-phosphate	UV-VIS	146
	TMOS	[Enz]-silica hydrogels (Monoliths, assays in presence of NADP)	Glucose-6-phosphate	Fluorescence	161

Table 3 (Continued)

Enzyme(s)	Precursors	Sensor Configuration	Analyte(s)	Sensor Type	Ref
Alcohol Dehydrogenase	TMOS	[Enz + NADH]-silica xerogels (Monoliths, liquid phase assays in buffer or hexane, or gas phase assays with alcohol vapour or gasoline-alcohol vapour)	Ethanol, 1-Propanol, Acetaldehyde, Propionaldehyde	Fluorescence	162
Nitrate Reductase	TMOS	[Enz]-silica hydrogels (Monoliths, biosensor reduced with dithionite prior to assay)	Nitrate	UV-VIS	163
Nitrite Reductase	TMOS	[Enz]-silica hydrogels and xerogels (Monoliths, and thin films sandwiched between silica xerogel thin films, on silica)	Nitrite	UV-VIS	164

[a] Abbreviations: 3-APTMOS, 3-aminopropyltrimethoxysilane; 3-GAPS, 3-gluconamidopropylsiloxane; 3-HEAGS, 3-(1′-hydroxy-2′-(2″-hydroxyethylamino)ethoxy)-propylglyceroxysiloxane; MeTEOS, methyltriethoxysilane; MeTMOS, methyltrimethoxysilane; 3-MPTMOS, 3-mercaptopropyltrimethoxysilane; PGS, poly(glyceryl silicate); TEOS, tetraethoxysilane; TMOS, tetramethoxysilane.

biological materials, together with the ability to produce optical and conducting sol−gels with diverse chemistries and fabricate them in a variety of formats.

Most work on sol−gel biosensors has focused on glucose sensors based around the flavoprotein glucose oxidase (GOx), which mediates the air oxidation of glucose to gluconolactone with concomitant generation of hydrogen peroxide (Table 2).[101−133] The enzyme has been encapsulated in silica, metal oxides, metallosilicates, Ormosils, and composite sol−gels, and the materials have been fabricated as monolithic, passive-deposited thin- and thick-film and spin-coated optical sensors and monolithic and passive-deposited thin- and thick-film electrodes and electrode coatings. Optical sensors monitor GOx function via UV−vis or fluorescence analysis of the chromophoric flavin cofactor, while electrochemical configurations can measure oxygen levels via a Clarke electrode or oxygen-sensitive rhodium(II)/(III) complexes, or monitor the redox cycling of the active site of GOx via its electrochemical coupling to an electrode surface using a dye or metallocene electron transfer mediator. Alternatively, the active site of GOx can be directly "wired" to an electrode by modification with a ferrocene derivative or via the codispersion/copolymerization of dye, transition metal complex, or metallocene redox mediators in the sol−gel and the activity monitored amperometrically.[102,103,111,116,119−122]

One of the most significant developments in amperometric GOx biosensors has been the advent of carbon composite electrodes based upon the incorporation of graphite together with mediators into GOx−Ormosil sol−gel compositions to provide highly sensitive and robust wired configurations, which can be mass-produced by screen printing.[102−104,112,113,119,122,127,131−133] The carbon phase provides electrical conductivity, porosity, and mechanical reinforcement, while the Ormosil sol−gel framework provides modifiable chemical functionality for fine-tuning the catalytic properties and stability of GOx as well as controlling the hydrophobicity and wetting behavior of the composite. This configuration enables the surface of monolithic and thick-film electrodes to be periodically renewed by simple polishing, to expose a fresh biosensor surface, once biosensor performance declines because of surface fouling and/or enzyme inactivation. In further modification, the inclusion of electrocatalytically active graphite-supported palladium, platinum, and ruthenium, or gold nanocrystals in place of graphite, provides mediator-free electrodes that are highly sensitive, selective, and stable.[103,105,111,125,128,130] Mediator-free amperometric configurations can also be constructed by coupling GOx with horseradish or soybean peroxidases, which mediate the reduction of hydrogen peroxide.[101,103,111−113,127] Alternatively, the bi-enzyme systems can be used to oxidize suitable dye precursors with colorimetric monitoring.[39]

The outstanding flexibility of sol−gel platforms to optical and amperometric biosensing has been amply demonstrated with its extension to a wide variety of oxidoreductases ranging from peroxidases, alcohol, amine, polyol and hydroxyacid oxidases and dehydrogenases, phenol oxidases, and hydroxylases to nitrite and nitrate reductases (Table 3).[134−164] The biosensors have been used for the monitoring of hydrogen peroxide, aliphatic alcohols and aldehydes, sugars, lactate, amines, phenols, organophosphates, competitive enzyme inhibitors such as cyanide and organophosphates, nitrates, nitrites, and polar organic solvents.

Although optical and amperometric configurations are readily constructed for chromoproteins and oxidoreductases, other proteins can also be applied, providing that the substrate/product can be monitored optically/electrochemically. Thus, the sol−gel-entrapped hydrolases cholinesterase, acetylcholinesterase, butyrylcholinesterase, phosphodiesterase, urease, and penicillinase have been used as direct and inhibition-based biosensors for the optical and potentiometric monitoring of acetylcholine, organophosphate pesticides, penicillin, and urea (Table 4).[165−172] Particularly interesting is the construction of coupled optical sensors employing bacteriorhodopsin together with urease, acetylcholine esterase, or penicillinase—the action of the hydrolase alters the pH and thus the protonation state of and hence the rate of decay of the photoactive M-state of bacteriorhodopsin.[172]

A variety of noncatalytic proteins that selectively bind metal ions and gaseous oxygen and oxides have also been utilized for optical sol−gel biosensing (Table 4). Thus, the luminescent protein has been used to construct sol−gel sensors for calcium, and the chromophoric metalloproteins hemoglobin, myoglobin, cytochrome *c*, and superoxide dismutase have been applied to the solution- and gas-phase detection/quantification of oxygen, carbon monoxide, carbon dioxide, and nitrogen monoxide. In particular, the heme proteins have proved to be excellent model systems for sol−gel bioencapsulation because the proteins are chromophoric, they can be de-readily metalated and metal-exchanged, and their conformation, molecular motions, and ligand binding can be easily monitored via spectroscopic probing of the active centers.[113,146,173−182]

Of particular interest to the food, drug, environmental, and medical fields is the fabrication of biosensors for detecting and quantifying antibody−antigen interac-

Table 4. Examples of Sol−Gel Optical, Electrochemical, and Immunochemical Biosensors Using Hydrolase, Ligand-Binding, and Photoactive Proteins[a]

Biological(s)	Precursor	Sensor Configuration	Analyte(s)	Sensor Type	Ref
Cholinesterase	TMOS	[Enz]-silica xerogel (Thick films on polymethacrylate, or thin films on silica optical fibres, inhibition assay with indoxyl acetate as substrate)	Fenitrothion, Azinphos-Ethyl, Methidathion, Naled, Mecarbam	Fluorescence	165
Butyrylcholinesterase	TMOS	[Enz]-silica xerogel (Thick films and monoliths, inhibition assay with indophenyl acetate as substrate)	BADAPP, Cognex	UV-VIS	166
Acetylcholinesterase Butyrylcholinesterase	TMOS	[Enz]-silica and [Enz]-Poly(ethylene glycol)-silica hydrogels (Thick films on polycarbonate microtiter plates, inhibition assays with acetylthiocholine and butyrylthiocholine as substrates)	Omethoate, Carbaryl, Diazinon, Malathion, Methamidophos, Chlorpyrifos, Methidathion	UV-VIS	167
Parathion Hydrolase	TMOS	[Enz]-silica xerogel (Thick films and granulates)	Parathion	UV-VIS	168
Urease	TMOS	[Enz]-silica hydrogels (Thin film on a inter-digitated Pt or Au electrode arrays deposited on alumina or silicon)	Urea	Potentiometric	169, 171
	TEOS	[Enz]-silica hydrogel (Thin film on Pt electrode)	Urea	Potentiometric	170
Bacteriorhodopsin + Acetylcholine Esterase or Penicillinase or Urease	TMOS	[Enzymes]-silica hydrogels (Thick films, assays conducted by monitoring the decay of the photoexcited M state of bacteriorhodopsin)	Acetylcholine Penicillin Urea	UV-VIS	172
Aequorin	TMOS	[Enz]-silica hydrogels	Ca^{2+}	Luminescence	173
Cytochrome *c* Cytochrome *c′*	TMOS	[Prot]-silica xerogels and hydrogels (Thin and thick films, spun coatings and monoliths, gas and liquid phase sensing)	CO, O_2, NO	UV-VIS	15, 174-179
Hemoglobin	TMOS	[Prot]-silica xerogels and hydrogels (Thin and thick films, spun coatings and monoliths, gas and liquid phase sensing)	CO, O_2, NO	UV-VIS Fluorescence	15, 175, 180
Myoglobin Mn-Myoglobin	TMOS	[Prot]-silica xerogels and hydrogels (Thin and thick films, spun coatings and monoliths, gas and liquid phase sensing	CO, CO_2, O_2, NO	UV-VIS Fluorescence	15, 27, 175, 176, 181, 182
Cu-Zn-Superoxide Dismutase	TMOS PGS	[Prot]-silica hydrogels and xerogels (Thick films and monoliths)	CN^-	UV-VIS	15, 125
Polyclonal Antibodies to Fibrin D Dimer Antigens	TMOS	[Antibody-Fluorescein conjugates]-silica hydrogels and xerogels (Thin film on silica optical fibres)	Fibrin D Dimer Antigens	Fluorescence	183
Polyclonal Antibodies to Fluorescein	TEOS	[Antibody]-silica xerogels (Thin film between silica xerogel thin films on glass)	5-/6-Carboxy-4′,5′-dimethylfluorescein	Fluorescence	184
Polyclonal Antibodies to Fluorescein	TMOS	[Antibody]-silica hydrogels and xerogels (Thin films)	Fluorescein	Fluorescence	185
Polyclonal Antibodies to Nitroaromatics	TMOS	[Antibody]-silica hydrogels and xerogels (Thick films)	1,3-Dinitrobenzene, 2,6-Dinitrophenylhydrazine	UV-VIS	186
Monoclonal Anti-Atrazine Antibodies	TMOS	[Antibody]-silica xerogels (Thick films and granulates)	Atrazine	ELISA	187, 188
Immunoglobulin (IgG) Antigens	TEOS	[IgG]-hydroxypropylcellulose-graphite-silica xerogels (Screen printed thick films on ceramic, ELISA assays with Goat anti-Rabbit IgG antibody-alkaline phosphatase conjugates and phenyl / napthyl phosphate substrates)	Goat anti-rabbit IgG antibodies	Amperometric	189
Echinococcus Parasite Hydatid Cyst Antigens	TMOS	[Antigens]-silica hydrogels and xerogels (Thick films on polycarbonate microtiter plates, ELISA assays with anti-human IgG antibody-peroxidase conjugates and *o*-phenylenediamine as substrate)	Human sera anti-*Echinococcus* antibodies	UV-VIS	190
Leishmania Parasite Promastigiote Antigens	TMOS	[Antigens]-silica hydrogels and xerogels (Thick films on polycarbonate microtiter plates, ELISA assays with anti-human IgG antibody-peroxidase conjugates and *o*-phenylenediamine as substrate)	Human sera anti-*Leishmania* antibodies	UV-VIS	191
Bacteriorhodopsin	TMOS PGS HEAGS	[Prot]-silica hydrogels and xerogels (Thick films on polystyrene and silica)	Phototransduction	UV-VIS	192-196
Phycoerythrin Phycocyanin Allophycocyanin	TMOS	[Prot]-silica hydrogels and xerogels (Thick and thin films on silica and glass optical fibres)	Phototransduction	UV-VIS Fluorescence	197

[a] Abbreviations: 3-HEAGS, 3-(1′-hydroxy-2′-(2″-hydroxyethylamino)ethoxy)propyl-glyceroxysiloxane; PGS, poly(glyceryl silicate); TMOS, tetramethoxysilane.

tions. Monoclonal and polyclonal antibodies to fluorescein, nitroaromatics, and organophosphates have been encapsulated in silica sol−gels and used for the optical, electrochemical, and coupled ELISA detection of the corresponding antigens (Table 4).[183−188] The reverse concept, namely, the entrapment of antigens, has been used to develop optical biosensors for detecting the presence of antibodies to disease-causing parasities in human blood.[190,191]

Photoactive proteins have also been entrapped for envisaged application as solid-state optical devices and transducers. Thus, the membrane-associated proteins

bacteriorhodopsin, phycocyanin, allophycocyanin, and phycoerythrin have been entrapped in silica hydrogels and xerogels and shown to retain their proton-pumping and photoactive properties.[192−197]

An area of application which has been slower to realize, but which offers great commercial promise, is that of sol−gel biocatalysts (Table 5).[16−29] With the growing application of biological catalysts to synthetic chemistry and industrial processes has come the need to develop high-performance immobized biological catalysts that are efficient, are stable to long-term operations under a variety of operating conditions, and can be fabricated on the large scale.[198−201] Initial work in this arena was restricted to the encapsulation of model hydrolases such as trypsin and acid phosphatase, but little was done on synthetic applications until fairly recently. Reetz et al. first established the general practical utility of sol−gel immobilized biocatalysts with their studies into the encapsulation of lipase enzymes in Ormosils and composite sol−gels.[202−208] The maximal activity of many lipases, and phospholipases and cutinises, requires the operation of a lid mechanism, whereby the contact with a hydrophobic interface displaces a segment of the tertiary structure to fully expose the binding site, thereby "activating" the enzyme toward its substrate.[64] Reasoning that it might be possible to employ hydrophobic Ormosils to both encapsulate and activate lipases, Reetz et al. showed that a variety of lipases could indeed be stably activated and entrapped in micro-phase-separated poly(alkylsiloxanes), hybrid poly(alkylsiloxane)−poly(dimethylsiloxane) sol−gels, and magnetite−Ormosil composites.[202−208] Other groups have since extended this methodology to functional Ormosils and filled composites sol−gels, and it appears that the technique is generically applicable to lipases, phospholipases, and cutinises.[209−218] The method gives access to particulate and thick film bioimmobilizates, which display aqueous- and organic-phase activities of 60−130% and 140−1400% of those of the soluble enzymes. The biocatalysts have been used to catalyze the regio-, chemo-, and enantioselective hydrolysis, esterification, and transesterification of carboxylic acids, alcohols, and esters and the acylation of amines in aqueous and aqueous−organic media and organic solvents. Fluka offers these catalysts for laboratory-scale trials, and Novo Nordisk markets several poly(alkoxysiloxane)−lipase immobilizates for industrial catalysis.

Various other hydrolases have also been encapsulated in inorganic, Ormosil, hybrid, filled, and IPN sol−gels (Table 5). Esterase, serine-, cysteine-, and metalloproteases, α- and β-glycosidases, acid and alkaline phosphatases, phospholipases, and organophosphorus hydrolases have been successfully entrapped in hydrogels and xerogels and applied to the hydrolysis of model compounds and the synthesis of bioactive peptides, glycosides, oligosaccharides, lipids, and so forth.[146,168,219−223]

Particulate and thick-film sol−gel biocatalysts have also been fabricated for lyase including natural aldolases, aldolase catalytic antibodies, oxynitrilase, and ketoacid decarboxylases and applied to the asymmetric aldol condensation, hydrocyanation, and addition of aldehydes and ketones.[146,224,225] Sol−gel-immobilized oxidoreductases, including lipoxygenases, tyrosinases, the heme proteins cytochrome *c*, hemoglobin, myoglobin, and horseradish peroxidase, and alcohol and polyol oxidases and dehydrogenases have likewise been used for the synthesis of polyunsaturated fatty acid hydroperoxides, the hydroxylation of aromatics, oxidative polymerization of phenolics, the *S*-oxidation of sulfides, and the regio- and enantioselective oxidation of alcohols.[146,226,227]

The flexibility and power of sol−gel encapsulation is well-demonstrated with its application to the preparation of multienzyme biocatalysts (Table 5).[146,228] Thus, it has been shown that the co-entrapment of enzymes which catalyze consecutive reactions in sol−gels can lead to an enhancement in overall catalytic efficiency and productivity, presumably because of the proximity of catalytic centers resulting in the efficient transfer of reaction intermediates between enzymes, effectively enhancing their local concentration. Thus, a system of the six proteins sialic acid aldolase, myokinase, pyruvate kinase, pyrophosphatase, CMP-sialate synthase, and α-(2,6)-sialyl transferase has been trapped in Ormosil−metallosilicate matrixes and applied to the continuous synthesis of the bioactive oligosaccharide α-(2,6)-sialyl-*N*-acetyllactosamine.[146] Similarly, formate dehydrogenase, formaldehyde dehydrogenase, and alcohol dehydrogenase have been co-encapsulated in silica and used to convert carbon dioxide directly to methanol.[228]

Finally, although this review has focused on nanocomposite bioencapsulates containing proteins and poly(nucleic acids), one should also mention that micron-sized living bacterial, fungal, plant, and animal cells have also been successfully encapsulated in a viable state and used for biosensing, biotransformations, and secondary metabolite production.[17,21,29] Perhaps the most remarkable demonstration of the biocompatibility of the technique is that silica-hydrogel-encapsulated mouse pancreatic islets of langerhans can be transplanted into diabetic mice wherein they act as bioartificial organs and secret insulin for extended periods.[17,21]

The Future for Sol−Gel Bioencapsulation

The last 10 years has seen an enormous growth of interest in the application of sol−gel platforms to the preparation of nanocomposite bioencapsulates, primarily for sensor, diagnostic, and catalyst applications.[16−29] To date, sol−gel research has uncovered some remarkable features:

(a) Sol−gel bioencapsulation appears generic—a remarkably diverse range of enzymes, noncatalytic proteins, DNA, RNA, organelles, and living cells have been successfully encapsulated in their viable state.

(b) Although encapsulation within a polymer matrix necessarily modifies the functioning of the biomaterial, the native activity can be largely retained therein.

(c) Inorganic, hybrid, and composite materials with exotic physico chemistries can be utilized for bioencapsulation.

(d) Bioencapsulation enables the conversion of labile biological materials into reusable and physicochemically robust nanocomposites, which can be fabricated and manipulated using conventional sol−gel processing.

(e) Biomolecules encapsulated in sol−gel polymers are protected from biological degradation and are often considerably stabilized to chemical and thermal inactivation.

Table 5. Examples of Sol−Gel Biocatalysts[a]

Biological(s)	Precursor(s)	Biocatalyst Configuration	Reaction	Ref
Lipases: *Candida rugosa* *Candida antartica* *Candida lipolytica* *Aspergillus niger* *Aspergillus oryzae* *Aspergillus awamori* *Rhizomucor miehei* *Mucor javanicus* *Pseudomonas cepacia* *Pseudomonas fluorescens* *Penicillium roquefortii* *Rhizopus arrhizus* *Humicola lanuginose* Wheat Germ Porcine Pancreas	TMOS TEOS MeTMOS PrTMOS BuTMOS HxTMOS OcTMOS ODcTMOS PGS TMOS + MeTMOS TMOS + PrTMOS PrTMOS + 3-APTMOS TMOS + PDMS MeTMOS + PDMS TEOS + 3-MPTEOS PrGS + PDMS-PGS	[Enz]-siloxane xerogels [Enz-ferrite]-siloxane xerogels [Enz]-magnetite-siloxane xerogels [Enz]-poly(vinyl alcohol)-siloxane xerogels [Enz]-gelatin-siloxane xerogels [Enz]-graphite-siloxane xerogels [Enz]-phyllosilicate-siloxane xerogels (Monoliths, thick film coatings on polypropylene, sintered clay and sintered glass, passive and spin coated thin films on glass, and powders)	Hydrolysis and transesterification of glycerolipids Hydrolysis and transesterification of esters Esterification of alcohols and carboxylic acids Enantioselective hydrolysis of esters Enantioselective acylation of amines and alcohols (In aqueous, monophasic and biphasic aqueous-organic media, and low-water and anhydrous organic solvents)	146, 202-218
Pig Liver Esterase	PMeMS + PMZrS MeGS + PGZrS	[Enz]-siloxane xerogels (Thick films on sintered clay, and powders)	Enantioselective hydrolysis of esters (In aqueous, monophasic aqueous-organic media, and low-water organic solvents)	146
Proteases: Trypsin Proteinase K Carboxypeptidase Y α-Chymotrypsin Subtilisin	TMOS TEOS PGZrS VnGS + PGS MeGS + PGS GAPS + PGAlS	[Enz]-siloxane hydrogels and xerogels [Enz]-metallosilicate xerogels (Thick films on silica and sintered clay, and powders)	Hydrolysis of amides Hydrolysis of N-protected amino acid esters Transesterification of unprotected amino acid esters Synthesis of dipeptides and oligopeptides (In aqueous, monophasic aqueous-organic media, and low-water organic solvents)	146, 219
Glycosidases: Rice α-Galactosidase Almond β-Glucosidase *Sulfolobus* β-Glucosidase Bovine β-Glucuronidase	TMOS PMS PGS PGS + GAPS PDMS-PGS	[Enz]-siloxane hydrogels and xerogels [Enz]-alginate-silica hydrogels and xerogels (Thick films on sintered clay, and powders and beads)	Hydrolysis of model and natural β-glucosides Synthesis of alkyl and hydroxyalkyl α-galactosides Synthesis of sterol β-glucuronides (In aqueous, monophasic and biphasic aqueous-organic media, and low-water organic solvents)	146, 220, 221
Phospholipase D	PMeGS + PGZrS	[Enz]-siloxane hydrogels and xerogels (Thick films on cellulose, and powders)	Hydrolysis and transesterification of phospholipids (Aqueous and monophasic aqueous-organic media)	146
Acid Phosphatase	PMS PGS	[Enz]-silica hydrogels and xerogels (Thick films on cellulose, and powders)	Hydrolysis of phenyl phosphate (In aqueous and monophasic aqueous-organic media)	146
Pseudomonas Atrazine Chlorohydrolase	TMOS TMOS + MeTMOS	[Enz]-siloxane hydrogels and xerogels (Powders)	Hydrolysis of Atrazine (In aqueous media)	222, 223
Organophosphorus Hydrolases: *Pseudomonas* Paraoxonase *Pseudomonas* Parathion Hydrolase	TMOS PGS PDMS-PMS PDMS-PGS	[Enz]-siloxane hydrogels and xerogels (Thick films on silica, and powders)	Hydrolytic detoxification of Paraoxon and Parathion (Aqueous and monophasic aqueous-organic media)	146, 168
Aldolase Catalytic Antibodies (Abzymes)	TMOS	[Antibody]-silica xerogel (Powders)	Asymmetric Aldol condensation of aldehydes and ketones (Aqueous media)	224
Aldolases: Rabbit Muscle Aldolase Sialic Acid Aldolase	PMS PGS PGTiS	[Enz]-silicate hydrogels and xerogels (Thick films on silica, and powders)	Asymmetric Aldol condensations of aldehydes and ketones (Aqueous and monophasic aqueous-organic media)	146, 225
Almond α-Hydroxynitrile Lyase (Oxynitrilase)	PMS GAPS + PGS GAPS + PGS + MeGS	[Enz]-siloxane hydrogels and xerogels (Thick films on silica, or powders)	Hydrolysis of α-hydroxynitriles Asymmetric hydrocyanation of aldehydes (In aqueous and monophasic aqueous-organic media)	146
Pyruvate Decarboxylase	PGAlS	[Enz]-silicate hydrogels and xerogels (Thick films on cellulose, and powders)	Synthesis of phenylacetyl carbinol (Aqueous and monophasic aqueous-organic media)	146
Soybean Lipoxygenase I	TMOS PMS MeGS + PGS	[Enz]-siloxane hydrogels and xerogels [Enz]-alginate-silica hydrogels and xerogels [Enz]-phyllosilicate-silica xerogels (Thick films on silica and sintered clay, powders, and beads)	Hydroperoxidation of linoleic and linolenic acids (Aqueous media)	146, 226
Mushroom Tyrosinase (Polyphenol Oxidase)	PMS PGS + GAPS	[Enz]-siloxane hydrogels and xerogels (Thick films on silica, and powders)	Hydroxylation of phenols and tyrosine derivatives (Aqueous media and low-water organic solvents)	146
Heme Proteins: Cytochrome *c* Hemoglobin Myoglobin Horseradish Peroxidase	TMOS PMS PGS	[Enz]-silica hydrogels and xerogels (Thick films on silica and sintered clay, and powders)	Oxidative polymerization of phenols *S*-Oxidation of dibenzothiophene *S*-Enantioselective sulfoxidation of sulfides (Aqueous and monophasic aqueous-organic media)	146, 227
Alcohol Oxidoreductases: Glucose Oxidase Glycerol-3-phosphate Oxidase Alcohol Dehydrogenase	PMS PGS PGZrS PMeMS + PMS PMeGS + PGS	[Enz]-siloxane hydrogels and xerogels (Thick films on silica and sintered clay, and powders)	Oxidation of glucose to gluconate Oxidation of glycerol-3-phosphate to glyceraldehyde-3-phosphate Enantioselective oxidation of primary alcohols Asymmetric reduction of aldehydes (Aqueous and monophasic aqueous-organic media)	146
Sialic Acid Aldolase + Sialyl Transferase + Myokinase + Pyruvate Kinase + CMP Sialate Synthase + Pyrophosphatase	3-APGS + PGZrS	[Enzymes]-siloxane xerogel (Thick film on silica, and powder)	Synthesis of the bioactive sialylated trisaccharide α-(2,6)-sialyl-*N*-acetyllactosamine (Aqueous media)	146
Formate Dehydrogenase + Formaldehyde Dehydrogenase	TMOS	[Enzymes]-silica hydrogels (Monoliths)	Conversion of carbon dioxide to methanol (Aqueous media)	228

Table 5 (Continued)

[a] Abbreviations: 3-APGS, 3-aminopropylglyceroxysilane; 3-APTMOS, 3-aminopropyltrimethoxysilane; BuTMOS, butyltrimethoxysilane; 3-GAPS, 3-gluconamidopropylsiloxane; 3-HEAGS, 3-(1′-hydroxy-2′-(2″-hydroxyethylamino)ethoxy)propylglyceroxysiloxane; HxTMOS, hexyltrimethoxysilane; MeGS, methylglyceroxysilane; MeTEOS, methyltriethoxysilane; MeTMOS, methyltrimethoxysilane; 3-MPTMOS, 3-mercaptopropyltrimethoxysilane; OcTMOS, octyltrimethoxysilane; ODcTMOS, octadecyltrimethoxysilane; PDMS, silanol-terminated poly(dimethylsiloxane); PGAlS, poly(glyceryl aluminosilicate); PGS, poly(glyceryl silicate); PGZrS, poly(glyceryl zirconosilicate); PMS, poly(methyl silicate); PMeGS, poly(methylglyceroxysilane); PMeMS, poly(methylmethoxysilane); PMZrS, poly(methyl zirconosilicate); PrTMOS, propyltrimethoxysilane; TEOS, tetraethoxysilane; TMOS, tetramethoxysilane; VnGS, vinylglyceroxysilane.

(f) Sol−gel bioencapsulates can be interfaced with spectroscopic and electrochemical platforms to generate biosensing devices.

However, studies have also exposed substantial hurdles that need to be overcome for the widespread adoption of the technology:[16−29]

(a) The biocompatibility of sol−gel protocols needs to be refined: although advances have been made with alcohol-free solutions and glyceroxysilane precursors, difficulties are still encountered with certain classes of biological, especially oligomeric biomolecules, membrane proteins, organelles, and live cells.

(b) Pore collapse during xerogel preparation remains a major issue: as well as being the single most important cause of bioactivity decline upon encapsulation, this can also lead to structural defects in films and monoliths and complicate fabrication procedures. Although some amelioration can be achieved via the application of special drying regimens, drying control additives, templating and pore-forming compunds, and glyceroxysiloxane precursors, a generic, practical, and scalable method for minimizing structural collapse has as yet to be devised.

(c) The porosity of current generation bioencapsulates is not optimal: low pore size and volume and the presence of a disordered/semicontinuous pore structure give rise to biomolecule subpopulations which are inaccessible to analytes, reduce biomolecule dynamics, and inhibit functioning and cause internal diffusional limitations. The availability of ordered mesocellular materials with large pore dimensions above 10 nm would considerably ease the fabrication of efficient materials for fast-biosensing and high-throughput biocatalysis. Most likely, this could be achieved via the application of structure-directing surfactants and polymers and the use of self-templating precursors.[40−52]

(d) Present day bioencapsulates generally show poor mechanical attributes: brittleness and a low resistance to mechanical stress are typical, and this poses a limit on many bulk applications. Unfortunately, here one is at odds with porosity—in general, the greater the pore size and volume, the more fragile and deformable the polymer is, and vice versa. A possible tradeoff is the synthesis of sol−gels with ordered mesopore or heirarchical pore assemblies, the honeycomb structures of which are known to enhance mechanical stability.[40−52] Alternatively, mechanical properties can be improved by using dendritic- or comb-type macromeric precursors, the application of IPN composites, and the use of reinforcing fillers, although this can also compromise porosity.[16−29,39]

Despite the many hurdles facing sol−gel bioentrapment, the rapid advances that have been made in the past decade in improving encapsulation protocols and diversifying applications have been remarkable.[16−29] Indeed, sol−gel nanoencapsulation offers the single most facile, generic, and promising methodology for the entrapment and stabilization of biological materials. Indeed, one can postulate some future directions for sol−gel nanocomposite bioencapsulates:

(a) Combinatorial discovery: recently developed methods for generating sol−gel polymer and heterogeneous catalyst libraries will be applied to the discovery of novel matrixes, the optimization of sol−gel compositions, and the screening of sensor and catalyst libraries for particular applications.[229,230]

(b) Bioencapsulation in transition metal alkoxides: the use of bulk transition metal oxides and co-encapsulated oxometalates and metal oxide colloids with conducting, catalytic, and/or chromic properties[1−3,21] may pave the way to novel bio-doped electro- and photocatalytic and electrochromic devices for novel biosensensors, photobioelectronics, and so forth.[231−237]

(c) Engineering of Ormosils: the appendage of moieties which can be directly wired to the active centers of oxidoreductases and photoproteins and interfaced with co-encapsulated conducting or photoactive polymers would enable the construction of integrated electrosensor and optical devices.[16−29] Similarly, the adaptation of strategies for the co-entrapment of transition metal catalysts will allow the execution of multicatalytic reaction cascades.[238−240]

(d) Advanced hybrids and composites: novel organic−inorganic sol−gels, IPN and filled composites, and multilayered architectures are expected to contribute to the development of highly responsive and rugged biosensors and biocatalysts that can be used in extreme environments and self-supporting catalytic membranes as well as enable the use of novel fabrication methods.[241,242]

(e) Templated sol−gels: the development of biocompatible templating methods based upon self-assembling mercaptosiloxanes and long-chain alkylsiloxanes, polyblock siloxane copolymers, and exogenous pore-forming additives should give access to encapsulates with highly ordered pore morphologies and chemical functionalities.[46−52] Furthermore, the deployment of molecular imprinting techniques should allow the addition of highly specific recognition functions to sol−gel biosensors and thereby improve selectivity, response, and resistance to interference and fouling.[243−248]

(f) Microfabrication methods: recently developed microstamping, soft lithography, microspotting, and inkjet printing technologies[249−251] are expected to be applied to sol−gel bioencapsulates to the fabrication arrays for drug screening, genomics, proteomics, and combinatorial synthesis[252−256] and the production of microsensors, microreactors, and bioelectronic devices.[257−261]

Although still in its infancy, the realm of sol−gel nanocomposite bioencapsulates offers to significantly advance a range of disciplines which interface with biology, from the production of biosensors, biocatalysts, and bioartificial organs to the fabrication of high-density bioarrays and bioelectronic devices. Indeed, it is ex-

pected that the coming years will witness the realization of a variety of research and industrial applications, especially those aimed at the catalysis, sensing/monitoring, diagnostics, biotechnology, and biocomputing sectors.

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