Encapsulation of Biologicals within Silicate, Siloxane, and Hybrid Sol−Gel Polymers: An Efficient and Generic Approach

Iqbal Gill*,†,‡,§ and Antonio Ballesteros†

Contribution from the Department of Biocatalysis, CSIC Institute of Catalysis, Campus Universidad Autonoma, 24049 Madrid, Spain, and FMS Department, BBSRC Institute of Food Research, Earley Gate, Whiteknights Road, Reading, Berkshire RG6 6BZ, U.K.

Received April 27, 1998

Abstract: The sol−gel encapsulation of labile biological materials with catalytic and recognition functions within robust polymer matrices remains a challenging task, despite the considerable research that has been focused on this field. Herein, we describe a new class of precursors, based around polyol silicates and polyol siloxanes, especially those derived from glycerol, that addresses problems faced with traditional bioencapsulation protocols. Poly(glyceryl silicate) (PGS) was prepared and employed for sol−gel bioentrapment, in an approach distinguished by a high biocompatibility and mild encapsulation conditions, and which enables the reproducible and efficient confinement of proteins and cells inside silica. The methodology was extended to metallosilicate, alkylsiloxane, functionalized siloxane, and composite sol−gels, thereby allowing the fabrication of a physicochemically diverse range of bio-doped polymers. The hybrid materials display activities approaching those of the free biologicals, together with the high stabilities and robustness that characterize sol−gel bioceramics. Indeed, the bioencapsulates performed better than those fabricated from tetramethoxysilane, poly(methyl silicate) or alcohol-free poly(silicic acid), even when the latter were doped with glycerol. The activity enhancements appear to derive at least in part from the unusual microstructure of PGS sol−gels, in particular their high porosity, although the underlying mechanisms are unclear. Differences in precursor hydrolysis/condensation, development of gel structure, biological-matrix interactions, precursor toxicity, and pore collapse probably all contribute to the observed efficiency of the PGS materials. The performances of the encapsulates are compared with conventional sol−biogels and other immobilizates, in representative biocatalyst, biosensor, and biodiagnostic applications.

Introduction

Developments over the last two decades in sol−gel processing have demonstrated the unique advantages offered by this technology in the materials science domain for the fabrication of optics, sensors, catalyst supports, coatings, and specialty polymers.[1] The striking extension of this technology to the entrapment of functionally active proteins in silica demonstrated that it is possible to introduce and retain labile biological activity within rugged glasslike polymers.[2] This realization, together with the flexibility to fabricate the resulting bioceramics in various forms including monoliths, thin films, powders, and fibers, has made these unique hybrid materials attractive for demanding applications in bioorganic synthesis, medicine, biotechnology, and environmental technology.[1c,2,3] As a result, vigorous efforts have been directed toward developing generic sol−gel bioencapsulation techniques for the efficient immobilization of sensitive biologicals, especially proteins and whole cells, within physicochemically robust inorganic and composite matrices.[1c,3] Indeed, to date, enzymes, catalytic antibodies, noncatalytic proteins, poly(nucleic acids), and microbial, plant, and animal cells have been employed for applications encompassing biocatalysts,[3a,c,d,k,4] biosensors,[3,5]

† CSIC Institute of Catalysis.

‡ BBSRC Institute of Food Research.

§ Present address: Biotechnology Department, Corporate R&D Division, Firmenich SA, CH-1211 Geneva 8, Switzerland. E-mail: iqbal.gill@firmenich.com.

(1) (a) Hench, L. L.; West, J. K.*Chem. Rev.* **1990**, *90*, 33. (b) Mark, J. E. *Heterog. Chem. Rev.* **1996**, *3*, 307. (c) Avnir, D. *Acc. Chem. Res.* **1995**, *28*, 328. (d) Hüsing, N.; Schubert, U. *Angew. Chem., Int. Ed. Engl.* **1998**, *37*, 22.

(2) (a) Braun, S.; Rappoport, S.; Zusman, R.; Avnir, D.; Ottolenghi, M. *Mater. Lett.* **1990**, *10*, 1. (b) Ellerby, L. M.; Nishida, C. R.; Nishida, F.; Yamanaka, F. A.; Dunn, B.; Valentine, J. B.; Zink, J. I. *Science* **1992**, *255*, 1113.

(3) (a) Avnir, D.; Braun, S. *Biochemical Aspects of Sol−Gel Science and Technology*; Kluwer: Hingham, MA, 1996. (b) Livage, J. *C. R. Acad. Sci. Paris* **1996**, *322*, 417. (c) Avnir, D.; Braun, S.; Lev, O.; Ottolenghi, M. *Sol−Gel Optics*; Klein, L., Ed.; Kluwer Academic: Berlin, 1994; pp 539−582. (d) Avnir, D.; Braun, S.; Lev, O.; Ottolenghi, M. *Chem. Mater.* **1994**, *6*, 1605. (e) Dave, B. C.; Dunn, B.; Valentine, J. S.; Zink, J. I. *Anal. Chem.* **1994**, *66*, 1120A. (f) Dave, B. C.; Dunn, B.; Valentine, J. S.; Zink, J. I. *ACS Symp. Ser.* **1996**, *622*, 351. (g) Lin, J.; Brown, C. W. *Trends Anal. Chem.* **1997**, *16*, 200. (h) Barreau, S.; Miller, J. N. *Anal. Commun.* **1996**, *33*, 5H. (i) MacCraith, B. D.; McDonagh, C. M.; O'Keeffe, G.; McEvoy, A. K.; Butler, T.; Sheridan, F. R. *Sens. Actuators, B* **1995**, *B29*, 51. (j) Zink, J. I.; Yamanaka, S. A.; Ellerby, L. M.; Valentine, J. S.; Nishida, F.; Dunn, B. *J. Sol-Gel Sci. Technol.* **1994**, *2*, 791. (k) Armon, R.; Dosoretz, C.; Starosvetsky, J.; Orshansky, F.; Saadi, I. *J. Biotechnol.* **1996**, *51*, 279.

(4) (a) Shtelzer, S.; Rappoport, S.; Avnir, D.; Ottolenghi, M.; Braun, S. *Biotechnol. Appl. Biochem.* **1992,** *15*, 227. (b) Reetz, M. T.; Zonta, A.; Simplekamp, J. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 301. (c) Pope, E.; Braun, K.; Van Hirtum, M.; Peterson, C. M.; Tresco, P.; Angrade, J. D. *Ceramic Trans.* **1995**, *55*, 33. (d) Pope, E. J. A. *Sol-Gel Sci. Technol.* **1995**, *4*, 225. (e) Heichal-Segal, O.; Rappoport, S.; Braun, S. *Biotechnology* **1995**, *13*, 798. (f) Campostrini, R.; Carturan, G.; Caniato, R.; Piovan, A.; Filippini, R.; Innocenti, G.; Cappelletti, E. M. *J. Sol-Gel Sci. Technol.* **1996**, *7*, 87. (g) Rietti-Shati, M.; Ronen, D.; Mandelbaum, R. T. *J. Sol-Gel Sci. Technol.* **1996**, *7*, 77. (h) Reetz, M. T.; Zonta, A.; Simpelkamp, J.; Koenen, W. *Chem. Commun.* **1996**, 1397. (i) Inama, L.; Dire, S.; Carturan, G.; Cavazza, A. *J. Biotechnol.* **1993**, *30*, 197. (j) Braun, S.; Rappoport, S.; Zusman, R.; Avnir, D.; Ottolenghi, M. *Mater. Lett.* **1990**, *10*, 1. (k) Shabat, D.; Grynszpan, F.; Saphier, S.; Turniansky, A.; Avnir, D.; Keinan, E. *Chem. Mater.* **1997**, *9*, 2258.

S0002-7863(98)01456-5 CCC: $15.00
Published on Web 08/15/1998

immunodiagnostics,[3,6] biooptical devices,[3,7] bioimplants and artificial organs,[3b,8] adsorbents for the removal of enzymes,[9] and the biosynthetic pathway stabilization.[10]

Despite the potential of this technology in the bioencapsulation arena, several complications derived largely from the alkyl silicate and alkoxyalkylsilane precursors employed for generating sol−gel matrices have restricted its wider utilization.[1c,3] The low water solubility and reactivity of these compounds typically necessitates cosolvents and catalysts, respectively, both of which can adversely affect the biological material of interest. Also, hydrolysis liberates alcohols, which are deleterious to bioactivity, and their resulting evaporation generates large-scale shrinkages and pore collapse during xerogel formation. Such complications together with difficulties encountered in controlling aging effects have meant that the reproducible production of stable, high-activity bioencapsulates has so far proved elusive.[1c,3] Here we describe an approach based around a novel class of biocompatible precursors, namely polyol esters of silicates and siloxanes, which addresses many of the above difficulties and permits the effective and reproducible fabrication of a diverse range of bio-doped sol−gel polymers.

Results and Discussion

We reasoned that the drawbacks of current sol−gel bioencapsulation protocols could be surmounted if one could prepare biocompatible sol−gel substrates displaying the following attributes: (i) a high water solubility; (ii) autohydrolysis in aqueous media; (iii) hydrolysis that would liberate a nonvolatile, bioprotective alcohol, which would also function as a drying control additive (DCA).[1] Polyol silicates were perceived as ideal candidates,[11] and after trials with various polyhydroxylated compounds,[12] glycerol was selected for its high biocompatibility and functionality. In addition, it was decided to utilize polysilicate esters because of their higher solids content, the reduced tendency of the produced sol−gels toward aging phenomena, and the greater robustness of the resultant xerogels.[1,13] Thus, the poly(glyceryl silicate) "$SiO_{1.2}Glc_{0.8}$" (PGS) was prepared as a stable, water-soluble solid 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 in a matter of minutes without the need for any catalyst, to form silica hydrogels, which after aging, washing to remove glycerol, and drying, produced transparent, mesoporous, and physically stable silica xerogels (Table 1).[14] Importantly, polymerization in the presence of proteins and cells allowed their efficient entrapment with surprisingly little loss of activity, to yield bioactive silica glasses. The results obtained with this approach in the encapsulation of representative proteins and cells are compared in Table 2 with those of a protocol employing PMS as precursor. It should be stressed that the entrapment of trypsin (Table 1) and α-chymotrypsin in sol−gels synthesized from alcohol-free poly(silicic acid) (PSA) solutions[4l] gave results similar to those obtained with PMS, despite the presence of 20−34% v/v of methanol in the latter hydrogels. In view of the denaturing effects of alkoxysilanes and alcohols on proteins,[3−5] we attempted to extend the PSA method to other biologicals, but in several cases (e.g., thermolysin, lipoxygenase, sialic acid aldolase, tyrosinase, and *S. salmonicolor* cells), poor results were obtained due to protein precipitation, premature/partial gelation, and/or hydrogel synerisis, especially at the higher PSA concentrations. Because of this, and the greater ease of preparation and handling and higher stability of PMS over PSA, the former was utilized as the precursor for the standard sol−gels. However, it is entirely possible that better activities could be obtained with the biologicals used in this study by using a suitably optimized PSA method, especially in the cases of whole cells which can be rather sensitive to alkoxysilanes and lower alcohols.

Properties of PGS-Derived Silica Matrices. Several notable features of the PGS approach can be appreciated. First, the mild encapsulation chemistry and high precursor biocompatibility significantly reduce toxicity effects, with the biogels retaining 83−98% of the activity of the native biological,[15] as compared with 11−76% for the PMS procedure (Table 2). In this context, one should note the moderate, but notable,

(5) (a) Braun, S.; Shtelzer, S.; Rappoport, S.; Avnir, D.; Ottolenghi, M. *J. Non-Cryst. Solids* **1992**, *147/148*, 739. (b) Yamanaka, S. A.; Nishida, F.; Ellerby, L. M.; Nishida, C. R.; Dunn, B.; Valentine, J. S.; Zink, J. I. *Chem. Mater.* **1992**, *4*, 495. (c) Pankratov, I.; Lev, O. *J. Electroanal. Chem.* **1995**, *393*, 35. (d) Yamanaka, S. A.; Dunn, B.; Valentine, J. S.; Zink, J. I. *J. Am. Chem. Soc.* **1995**, *117*, 9095. (e) Wang, J.; Pamidi, P. V. A.; Park, D. S. *Anal. Chem.* **1996**, *68*, 2705. (f) Wang, R.; Narang, U.; Prasad, P.; Bright, F. V. *Anal. Chem.* **1993**, *65*, 2671. (g) Sampath, S.; Pankratov, I.; Gun, J.; Lev, O. *J. Sol-Gel Sci. Technol.* **1996**, *7*, 123. (h) Akbarian, F.; Lin, A.; Dunn, B. S.; Valentine, J. S.; Zink, J. I. *J. Sol-Gel Sci. Technol.* **1997**, *8*, 1067. (i) Navas Dias, A.; Ramos Peinado, M. C. *Sens. Actuators* **1997**, *B39*, 426. (j) Coche-Guerente, L.; Cosnier, S.; Labbe, P. *Chem. Mater.* **1997**, *9*, 1348. (k) Wang, J.; Pamidi, P. V. A.; Park, D. S. *Electroanalysis* **1997**, *9*, 52. (l) Blyth, D. J.; Oytner, S. J.; Russell, D. A. *Analyst* **1996**, *121*, 1975. (m) Aylott, J. W.; Richardson, D. J.; Russell, D. A. *Analyst* **1997**, *122*, 77. (n) Yamanaka, S. A.; Nguyen, N. P.; Dunn, B.; Valentine, J. S.; Zink, J. I. *J. Sol-Gel Sci. Technol.* **1996,** *7*, 117. (o) Glezer, V.; Lev, O. *J. Am. Chem. Soc.* **1993**, *115*, 2533. (p) Blyth, D. J.; Aylott, J. W.; Richardson, D. J.; Russell, D. A. *Analyst* **1995**, *120*, 2725. (q) Narang, U.; Prasad, P. N.; Bright, F. V.; Kumar, A.; Kumar, N. D.; Malhotra, B. D.; Kamalasanan, M. N.; Chandra, S. *Chem. Mater.* **1994**, *6*, 1596. (r) Shtelzer, S.; Braun, S. *Biotechnol. Appl. Biochem.* **1994**, *19*, 293. (s) Dosoretz, C.; Armon, R.; Starosvetsky, J.; Rothschild, N. *J. Sol-Gel Sci. Technol.* **1996**, *7*, 7.

(6) (a) Barreau, J. Y.; Da Costa, J. M.; Desportes, I.; Livage, J.; Monjour, M.; Gentilini, M. *Compt. Rend. Acad. Sci., Ser. III* **1994**, *317*, 653. (b) Livage, J.; Barreau, J. Y.; Da costa, J. M.; Desport, I. *SPIE Proc.* **1994**, *2288*, 493. (c) Livage, J.; Roux, C.; Da Costa, J. M.; Desportes, I.; Quinson, J. F. *J. Sol-Gel Sci. Technol.* **1996**, *7*, 45. (d) Wang, R.; Narang, U.; Prasad, P. N.; Bright, F. V. *Anal. Chem.* **1993**, *65*, 2671.

(7) (a) Weetall, H. H. *Biosens. Bioelectr.* **1996**, *11*, 327. (b) Wu, S.; Ellerby, L. M.; Cohan, J. S.; Dunn, B.; El-Sayed, M. A.; Valentine J. S.; Zink, J. I. *Chem. Mater.* **1993**, *5*, 115. (c) Chen, Z.; Samuelson, L. A.; Akkara, J.; Kaplan, D. L.; Gao, H.; Kumar, J.; Marx, K. A.; Tripathy, S. K. *Chem. Mater.* **1995**, *7*, 1779. (d) Chen, Z.; Kaplan, D. L.; Yang, K.; Kumar, J.; Marx, K. A.; Tripathy, S. K. *J. Sol-Gel Sci. Technol.* **1996**, *7*, 99.

(8) (a) Pope, E. J. A.; Braun, K.; Peterson, C. M. *J. Sol-Gel Sci. Technol.* **1997**, *8*, 635. (b) Pope, E. J. A. *Ceram. Trans.* **1996**, *63*, 17.

(9) Narang, U.; Rahman, M. H.; Wang, J. H.; Prasad, P. N.; Bright, F. V. *Anal. Chem.* **1995**, *67*, 1935.

(10) Bressler, E.; Braun, S. *J. Sol-Gel Sci. Technol.* **1996**, *7*, 129.

(11) Although additives such as PEG and PVA have been included in sol−gel procedures,[1a−c] to our knowledge, the polyol ester precursors presented here have not been previously reported, nor have similar compounds been utilized for bioencapsulation purposes.

(12) Silicate and polysilicate esters of ethylene glycol, propylene glycol, diglycerol, triethylene glycol, etc., were readily accessed via transesterification.

(13) Ellsworth, M.; Novak, B. M. *Chem. Mater.* **1993**, *5*, 839.

(14) FTIR analysis indicated complete hydrolysis, with no trace of glycerol being observed in the xerogels. Also, the monoliths were found to be mechanically and optically stable to repeated cycles of wetting with aqueous or organic media, followed by drying.

(15) Direct evidence for the diffusion of substrates into xerogels and their reaction with the internalized enzymes was obtained by incubating monoliths with chromogenic substrates. Thus, when silicas containing *C. rugosa* lipase, β-glucosidase, and horseradish peroxidase were respectively contacted with aqueous solutions of 4-nitrophenyl palmitate, 4-nitrophenyl β-D-glucopyranoside or hydrogen peroxide, and 1,4-hydroquinone, the generation of colored products and accompanying color development was observed throughout the sol−gel matrix. Control reactions where xerogels were placed in solutions of the products were used to distinguish the simple diffusion of these compounds into the gel interiors.

Table 1. Comparison of PGS-, PMS-, and PSA-Derived Silica Sol−Gels Doped with Trypsin

precursor	H_2O/Si (w/w)[a]	sol−gel material[b]	gel time (min)	pore size (nm)[c]	pore volume (mL/g)	shrinkage (% v/v)[d]	encapsulation (%)[e]	activity (%)[f]
PGS	3	aged hydrogel	3.6	11.8	2.202	4	99	98
	3	xerogel		10.4	1.945	16	97	97
	9	aged hydrogel	2.7	13.8, 16.4[g]	2.760	29	98	98
	9	xerogel		11.5, 13.2[g]	2.228	40	95	95
PMS + G	3	aged hydrogel	4.6	4.7	1.185	39	98	41
	3	xerogel		4.5	0.963	47	96	36
	9	aged hydrogel	3.8	7.5, 8.9[g]	1.618	53	97	43
	9	xerogel		7.1	1.139	61	95	40
PMS	3	aged hydrogel	4.2	3.3	0.714	67	99	34
	3	xerogel		3.1	0.690	73	98	31
	9	aged hydrogel	3.5	6.8	0.672	76	96	36
	9	xerogel		6.7	0.636	83	94	29
PSA + G	9	aged hydrogel	13	7.4, 9.2[g]	1.543	47	96	47
	9	xerogel		6.8, 7.5[g]	1.028	51	94	45
PSA	9	aged hydrogel	17	7.1	0.695	73	97	40
	9	xerogel		6.9	0.620	79	96	34

[a] Water:Si molar ratio used in preparation. [b] The aged hydrogel was obtained by aging the fresh sol−gel in a closed container at 5 °C for 20 h, then drying in air at 20 °C for 96 h. Washing with 2 × 20 volumes of phosphate (50 mM, pH 7.5, 20 °C, 24 h), then freeze-drying at 20 °C, 72 h gave the xerogel. [c] Mean pore size from TPM, corresponding to 50% of the pore volume. The pore distributions extended over a range of ±1.3 to ±1.7 nm. [d] Relative to the initial volume of the sample, prior to gelation. [e] Overall percentage of protein retained in the sol−gel after washing with 2 × 20-fold volumes of phosphate (50 mM, pH 7, 20 °C, 5 h). [f] Activity of encapsulate (50−100 μm particles), in an aqueous assay with L-BAPNA, as compared with the free enzyme. [g] Bimodal pore radius distribution. Initial trypsin dopings of 30 mg/g of xerogel were used.

Table 2. Biologicals Encapsulated in Silica, Metallosilicate, and Alkylsiloxane Matrices; Polyol Silicate/Siloxane-Derived Bioencapsulates vs Standard Alkyl Silicate/Alkoxysiloxane-Based Immobilizates [a]

biological encapsulated	matrix composition[b]	H_2O/Si (w/w)[c]	loading (mg/g)[d]	encapsulation (%)[e]	relative activity (%)[f]
		Silica Sol−Gels			
α-chymotrypsin	SiO_2	6	10−100	95−98 *vs 64−93*	94−99 *vs 16−33*
superoxide dismutase	SiO_2	6	5−20	88−91 *vs 86−93*	91−93 *vs 67−74*
cytochrome *c*	SiO_2	6	10−30	93−94 *vs 93−97*	92−97 *vs 70−76*
α-galactosidase	SiO_2	6	5−25	91−95 *vs 88−96*	90−95 *vs 41−48*
bovine catalase	SiO_2	6	25	91 *vs 92*	93 *vs 37*
horseradish peroxidase	SiO_2	6	10−50	83−95 *vs 76−90*	79−94 *vs 31−49*
C. rugosa lipase	SiO_2	6	1−20	95−96 *vs 83−89*	86−99 *vs 48−56*
yeast cells	SiO_2	6	50200	88−96 *vs 56−83*	85−89 *vs 13−38*
		Metallosilicate Sol−Gels			
subtilisin	SiO_2:ZrO_2 (9:1)	6	10−100	92−99 *vs 73−99*	96−99 *vs 11−35*
rabbit muscle aldolase	SiO_2:TiO_2 (19:1)	4	10−25	95−99 *vs 91−94*	93−96 *vs 27−40*
glycerol 3-phosphate oxidase	SiO_2:ZrO_2 (19:1)	6	20	93 *vs 92*	88 *vs 43*
glucose oxidase	SiO_2:ZrO_2 (19:1)	6	1−15	89−93 *vs 86−93*	91−92 *vs 43−56*
yeast pyruvate decarboxylase	SiO_2:Al_2O_3 (19:1)	5	10−100	85−94 *vs 70−91*	83−98 *vs 11−45*
R. miehei Cells	SiO_2:ZrO_2 (9:1)	6	50−300	84−93 *vs 41−86*	83−87 *vs 9−37*
		Alkylsiloxane Sol−Gels			
thermolysin	$MeSiO_{1.5}$:SiO_2 (4:1)	3	40	93 *vs 78*	90 *vs 24*
horse liver alcohol dehydrogenase	$MeSiO_{1.5}$:SiO_2 (1:1)	4	20	96 *vs 92*	91 *vs 47*
soybean type 1 lipoxygenase	$MeSiO_{1.5}$:SiO_2 (9:1)	3	10−75	88−95 *vs 74−89*	84−93 *vs 16−48*
pig liver esterase	$MeSiO_{1.5}$:SiO_2:ZrO_2 (9:9:2)	3	25	91 *vs 86*	93 *vs 42*
phospholipase D	$MeSiO_{1.5}$:SiO_2:ZrO_2 (7:2:1)	3	10	92 *vs 89*	89 *vs 39*
P. oleovorans cells	$PrSiO_{1.5}$:SiO_2 (8:2)	3	50−300	87−90 *vs 53−86*	81−88 *vs 13−37*

[a] The first figure/range refers to encapsulates made using polyol precursors, and those in italics to the PMS and methyl/ethyl silicate ester-based immobilizates. [b] Xerogel composition (w/w). [c] Molar ratio of water to Si used for preparation. [d] Amount of biological (protein, wet cells, or dry spores) used for encapsulation, quoted per gram of xerogel after washing and drying. [e] Overall percentage of biocatalyst retained in sol−gel after washing with a 2 × 20-fold volumes of phosphate (50 mM, pH 7, 2−20 h). [f] Activity of encapsulate (50−100 μm particles) directly after preparation, in an aqueous assay as compared with the free biological. In all cases, compositionally equivalent materials were prepared by the standard protocol, using PMS and/or partially condensed alkoxyalkylsiloxanes.

improvement in activity upon switching from PMS to PSA as precursor (Table 1). The high aqueous solubility of PGS also means that immobilization is efficient, typically enabling 88−98% entrapment at loadings as high as 20% w/w of xerogel, without unduly compromising bioactivity or matrix integrity. In contrast, the hydrophobicity of alkyl silicates and the denaturing effects of the alcoholic cosolvents and byproducts usually limits loadings in conventional protocols to 0.1−5% w/w, due to the aggregation/precipitation of the biological at elevated concentrations. This reduces entrapment efficiency and bioactivity recovery at high dopings, whereas in the PGS route, such effects are rarely observed, by virtue of the excellent aqueous miscibility and biocompatibility of both PGS and glycerol.[16] The use of alcohol-free PSA solutions similarly enables high doping levels, although precipitation and partial gelation/synerisis were observed with several proteins including thermolysin, lipoxygenase, and pyruvate decarboxylase.

Hydrolysis and gelation of PGS is rapid in aqueous milieu and does not require the use of catalysts. Indeed, ^{29}Si NMR studies indicated that the hydrolysis and condensation rates of polyol silicates and polysilicates were 6−23 times higher than

(16) (a) Priev, A.; Almagor, A.; Yedgar, S.; Gavish, B. *Biochemistry* **1996**, *35*, 2061. (b) Rui, S. *Acta Crystallogr., Sect. D: Biol. Crystallogr.* **1995**, *D51*, 271. (c) Dooha, K.; Lee, Y. J. *J. Therm. Biol.* **1993**, *18*, 41.

those of TMOS and PMS in buffered media.[17] This presumably stems from the increased reactivity of the silicon center due to the electron-withdrawing effect of the polyol moieties, together with internally assisted hydrolysis by the hydroxyl functions.[1a,b] Interestingly, reactivity could be controlled to some extent by adjusting the precursor composition, with more rapid gelation being observed upon decreasing the polyol content or when substituting a diol silicate for a triol or higher polyol silicate.[18]

PGS-derived silicas form exceptionally porous matrices, with thermoporometric (TPM) analysis of trypsin-doped sol−gels indicating that the mesopore structure and pore volume of the hydrogels was substantially preserved upon processing to the xerogels (Table 1).[19] In comparison, considerable pore collapse was observed for the products derived from PMS or PSA with or without the inclusion of glycerol as modifier (Table 1). Although bio-doped silicas with pore sizes of up to 10 nm and pore volumes of 0.4−0.9 mL/g have been reported for hydrogels or xerogels prepared at high pHs,[5a,r,6b,c,8] most of the silica xerogels prepared to date have displayed pore diameters in the range of 3−5 nm and porous volumes below 0.7 mL/g.[3,20] Also, experiments on the poly-L-lysine inhibition of *N*-L-benzoyl-L-arginine-4-nitroanilide (L-BAPNA) hydrolysis by trypsin indicated a substrate exclusion limit between 7×10^4 and 1.5×10^5 for the PGS-derived glasses, as compared with about 3×10^4 for TMOS, PMS, and PSA xerogels.[4a,22] This is in accord with the previously reported ease of diffusion of antibodies with masses of 1.5×10^5 into antigen-doped silica hydrogels,[6b,c] and the binding of RNase by silica-encapsulated DNP-poly(nucleic acid) inhibitors.[9] Furthermore, the kinetic constants for L-BAPNA hydrolysis by PGS-encapsulated trypsin (k_{sp}* of 104 ± 21 mM/min, k_{sp}* is the apparent specificity constant) were very similar to those of the free enzyme (k_{sp} of 128 ± 21 mM/min), whereas the PMS- and PSA-encapsulates displayed substantially poorer kinetics (k_{sp}* of 61 ± 11 and 68 ± 9 mM/min, respectively), and similar results were obtained with alkaline phosphatase.[24] Analyses of the kinetics indicated homogeneous enzyme populations in all cases, rather than the heterogeneous activity distributions that have been observed for these enzymes trapped in TMOS silicas.[4a,j]

Thus, the microstructures of PGS sol−gels are quite different to that of the TMOS, PMS, or PSA products, even when an equivalent amount of glycerol is used in the latter cases. It is possible that differences in the hydrolysis and/or condensation mechanisms,[1d] and the seeding, evolution, and consolidation of the protein−silica interface and the polymer and pore frameworks during gelation and drying,[1,25] are responsible for the distinct features of the PGS sol−gels. Although the inclusion of glycerol in the PMS protocol did reduce pore collapse (Table 1), relatively little improvement in bioencapsulate activity was observed, in accord with the intrinsic toxic and aging effects associated with the use of alkyl silicates. As expected, an increased activity was seen for the alcohol-free PSA sol−gels (Table 1), although the rather moderate effects of glycerol inclusion on the pore structure and activity once again emphasized the singular attributes of PGS. The DCA properties of PGS are apparent from the much smaller shrinkages observed for PGS-xerogels (Table 1). Under typical encapsulation conditions, volume contractions of 10−17% were recorded, compared to 47−75% for the TMOS, PMS, and PSA silicas.[1c,3]

At the same time, the PGS-immobilizates showed the characteristically high resistance to leaching and the extended storage stabilities of sol−gel encapsulates.[3] Incubation of protein immobilizates prepared at H_2O/Si molar ratios of 3, 6, and 9, in 50 mM phosphate, pH 7, at 5 °C for 10 months resulted in 1−5%, 2−7%, and 6−13% of protein loss and the retentions of 93−99%, 91−99%, and 86−93% of the initial activity, respectively. This is in agreement with previous observations on undried and partially dried hydrogels.[21c,2b,3c,d,5a,r] The surprisingly low levels of leaching found for such porous solids may derive from the biomolecule-directed templating of the developing sol−gel matrix, resulting in the development of extensive protein−silica surface binding interactions, in addition to simple embedding in the inorganic framework.[3c,e] Interestingly, experiments with α-chymotrypsin, trypsin, *C. rugosa* lipase, and β-glucosidase showed that the higher levels of protein loss found with the silicas prepared at a H_2O/Si ratio of 9 were reduced to 1−4% upon the inclusion of 3-glycidoxy- or 3-(3′-epoxycyclohexyloxy)propyltrimethoxysilanes (2−5% w/w of xerogel) in the sol−gel protocols, thus introducing covalent matrix−biomolecule attachment sites. However, this improvement was obtained at the cost of the activity of the entrapped protein, which was reduced by 3−14% compared to the nonmodified protocol.

Another property of sol−gel protein encapsulates, namely elevated thermostability was also observed.[3,4a,j] Thus, the half-lives of PGS-immobilized (H_2O/Si ratio of 6) *C. rugosa* lipase, β-glucosidase, and acid phosphatase in aqueous buffer at 70 °C were increased by factors of 56, 32, and 94 over those of the free enzymes, approaching the 63-, 38-, and 126-fold enhancements observed for the PMS encapsulates.[26] It seems

(17) (a) Hook, R. J. *J. Non-Cryst. Solids* **1996**, *195*, 1. (b) Van Beek, J. J.; Seykens, D.; Jansen, J. B. H. *J. Non-Cryst. Solids* **1992**, *146*, 111.

(18) This may reflect an increase in condensation rates deriving from the reduced formation of internal, metastable diol−silanol complexes. The formation of polyol−silanol and alkylamino−silanol complexes in aqueous media is know to reduce silanol condensation rates[1a] and in extreme cases lead to gelation-resistant products.

(19) (a) Kazuhiko, I.; Minoru, T. *J. Colloid Interface Sci.* **1995**, *171*, 103. (b) Ishikiriyama, K.; Todoki, M.; Min, K.; Yonemori, S.; Noshiro, M. *J. Therm. Anal.* **1996**, *46*, 1177. (c) Ishikiriyama, K.; Todoki, M. *Thermochim. Acta* **1995**, *256*, 213.

(20) Although mesoporous silicas with pore diameters of up to 200 nm can be prepared from TMOS by the careful choice of catalyst, hydrolysis conditions, DCA type, and hydrogel processing,[1,20] the reported methods are too aggresive for bioencapsulation.

(21) (a) Sakka, S.; Kozuka, H.; Adachi, T. *Ceram. Trans.* **1993**, *31*, 27. (b) Xi, Y.; Zhang, L.; Wang, S. *Sens. Actuators, B* **1995**, *B25* (1−3), 347−352.

(22) The percentage inhibitions observed for poly-L-lysines of M_r (1−4) × 10^3, (4−15) × 10^3, (15−30) × 10^3, (3−7) × 10^4, (7−15) × 10^4, (15−30) × 10^4 were 11 ± 1%, 23 ± 3%, 47 ± 2%, 61 ± 5%, 53 ± 6%, and 41 ± 2%for the free enzyme, 33 ± 2%, 41 ± 5%, 16 ± 2%, 3 ± 0.1%, 2 ± 0.1%, and 1 ± 0.1% for the PMS-encapsulate, and 38 ± 5%, 45 ± 6%, 68 ± 3%, 49 ± 6%, 27 ± 4%, and 9 ± 1% for the PGS-encapsulate. These results are consistent with the TPM analyses if one assumes a globular random coil model for the inhibitors[23] and takes into account ionic interactions and adsorption effects between the inhibitor and the charged silica framework.

(23) Applequist, J.; Doty, P. *Polyamino Acids, Polypeptides and Proteins*; Stahman, M. A., Ed.; University of Wisconsin Press: Madison, WI, 1962; pp 161−176.

(24) The following kinetic parameters were observed for the trypsin-catalyzed hydrolysis of L-BAPNA: The free enzyme gave a k_{cat} of 123 ± 20 min^{-1} and a K_m of 0.96 ± 0.13 mM, the PMS-encapsulate a k_{cat}* of 71 ± 13 min^{-1} and a K_m* of 1.17 ± 0.08 mM, and the GPS-encapsulate a k_{cat}* of 109 ± 22 min^{-1} and a K_m* of 1.05 ± 0.16 mM. Alkaline phosphtase gave the following results for the hydrolysis of NPP: The free enzyme gave a k_{cat} of 534 ± 62 min^{-1} and a K_m of 0.11 ± 0.02 mM, the PMS-encapsulate a k_{cat}* of 260 ± 58 min^{-1} and a K_m* of 0.25 ± 0.03 mM, and the GPS-encapsulate a k_{cat}* of 461 ± 38 min^{-1} and a K_m* of 0.13 ± 0.01 mM.

(25) (a) Nakanishi, K. *J. Porous Mater.* **1997**, *4*, 67. (b) Parashar, V. K.; Raman, V.; Bahl, O. P. *J. Mater. Sci. Lett.* **1996**, *15*, 1403.

(26) The half-lives at 70 °C were as follows (times for free enzyme, GPS-encapsulate, and PMS-encapsulate, respectively): β-glucosidase (12.7 ± 0.8, 673 ± 51, and 756 ± 65 h), acid phosphatase (0.12 ±0.01, 11.3 ± 1.4, and 15.1 ± 1.3 min), and *C. rugosa* lipase (23 ± 1.8 min, 12.3 ± 0.9 h, and 14.6 ± 1.7 h).

Table 3. Biologicals Encapsulated in Functionalized Siloxane and Composite Matrices; Polyol Ester-Derived Functionalized Siloxane and Composite Bioencapsulates vs Standard TMOS-Based Immobilizates[a]

biological encapsulated	matrix composition[b]	H_2O/Si (w/w)[c]	loading (mg/g)[d]	encapsulation (%)[e]	relative activity (%)[f]
		Functionalized Siloxane Sol−Gels			
proteinase K	$CH_2CHSiO_{1.5}$:SiO_2 (5:1)	3	25−75	90−93 *vs 68−96*	89−95 *vs 21−39*
sialic acid aldolase	APS:SiO_2 (1:4)	5	5−15	93−97 *vs 89−94*	89−94 *vs 28−35*
bacteriorhodopsin	HEAS:SiO_2 (1:5)	3	1−5	91−94 *vs 90−91*	79−88 *vs 31−43*
aspartate aminotransferase	LPS: SiO_2 (2:3)	3	10−50	87−93 *vs 81−89*	88−92 *vs 23−40*
almond oxynitrilase	GAPS:SiO_2 (1:3)[g]	4	20−50	91−94 *vs 86−93*	87−93 *vs 26−44*
β-glucosidase	GAPS:SiO_2 (1:3)[g]	4	100	94 *vs 73*	96 *vs 31*
tyrosinase	GAPS:SiO_2 (1:3)[g]	4	20−50	84−89 *vs 79−86*	82−92 *vs 19−31*
glucose oxidase	FPS:SiO_2 (4:6)	3	1−5	87−90 *vs 88−92*	91−92 *vs 41−44*
carboxypeptidase Y	BHP:SiO_2 (1:4)	3	20	93 *vs 87*	93 *vs 35*
		Composite Sol−Gel Polymers			
β-glucuronidase	$PDMS_{1.7K}$:SiO_2 (1:1)	3	40	95 *vs 92*	91 *vs 41*
A. niger spores	$PDMS_{4.2K}$:SiO_2 (1:1)	3	50−250	91−94 *vs 83−91*	83−91 *vs 25−43*
penicillin acylase	Alginate:SiO_2 (1:10)	6	10−40	94−99 *vs 88−93*	87−92 *vs 27−39*
yeast cells	PVP: SiO_2 (3:7)	6	300	87 *vs 82*	83 *vs 32*
firefly luciferase	PGMA:SiO_2 (4:3)	6	10	87 *vs 91*	84 *vs 39*
S. salmonicolor cells	$PVA_{85-146K}$:SiO_2 (1:1)	6	50−300	85−98 *vs 68−89*	74−85 *vs 20−34*

[a−f] As for Table 1. [g] In the conventional procedure, PMS replaced GAPS. Abbreviations: APS, 3-aminopropylsiloxane; BHP, *O,O′*-bis(2-(2′-hydroxy-2′-(3″-oxysilylpropoxy)ethylamino)propyl)PEG_{500}; FPS, 3-ferroceneacetamidopropylsiloxane; HEAS, 3-(1′-hydroxy-2′-(2″-hydroxyethylamino)ethoxy)propylsiloxane; GAPS, 3-gluconamidopropylsiloxane; LPS, 3-leucinamidopropylsiloxane; PGMA, poly(glyceryl methacrylate); PDMS, poly(dimethylsiloxane); PVA, poly(vinyl alcohol).

difficult to reconcile the high activities which would suggest a considerable degree of biomolecule mobility, with increased thermostability, which implies substantial conformational restriction. However, fluorescence studies on labeled BSA and HSA trapped in silica xerogels have pointed to a high degree of protein mobility,[27] while large increases in thermostability have been described for encapsulated phosphatases and trypsin.[4a,j] This suggests that the proteins are trapped within cavities where the local biomolecule mobility is sufficiently high to preserve an aqueous-type activity when complemented by a highly porous matrix, but at the same time, the more extensive conformational changes that are required for thermal denaturation are hindered. The latter could derive from hydrogen bonding and ionic interactions at polymer−protein contact surfaces formed via templating[3a] and/or from the reduction in segmental mobility due to the increased viscosity of the trapped solvent as compared to bulk solvent.[27] The greater thermostabilities of the PMS-derived materials thus presumably reflect a more restrictive silica framework and/or a higher trapped solvent viscosity for these materials.

Encapsulation in Other Matrices. Although the xerogels obtained above provided highly active, optically transparent encapsulates, their practical application was limited by two significant drawbacks of silica sol−gel bioimmobilizates, namely their brittleness and lack of additional functionality.[2−5] To address these issues, as well as to examine the general applicability of the polyol ester approach, we then attempted to extend it to other matrices, using representative biologicals from the biocatalyst, biorecognition, and biosensor domains, for evaluation purposes. Metallosilicates which display a greater mechanical strength and chemical inertness, and alkylsiloxanes with their elevated hydrophobicity and fracture resistance, were made using poly(glyceryl metallosilicates) containing Ti, Zr, and Al and poly(glyceroxyalkylsiloxanes) carrying C_1−C_8 alkyl functionalities, respectively. Once again, biologicals were readily immobilized within the sol−gels, and the results obtained with the polyol-derived materials and compositionally identical products synthesized via the PMS/poly(alkyl metallosilicate) or PMS/poly(alkoxyalkylsilane) methodology are shown in Table 2. The metallosilicates furnished brittle, transparent (below 10−15% w/w of metal oxide), or translucent (above 15% w/w metal oxide) glasses. The alkylsiloxanes provided hard, transparent/translucent, mesoporous glasses at H_2O/alkylsiloxane ratios below about 0.4−0.6 w/w. At higher water contents, the hydrophobicity of the alkylsiloxanes coupled with the high aqueous solubility of their precursors led to phase separation during gelation, ultimately resulting in opaque, brittle solids or spongy-like polymers with 2−70 μm continuous macropore networks.[28]

Encouraged by these results, we then sought to introduce functional siloxanes with the aim of modulating hydrophilicity, surface interactions, substrate partitioning, etc., which are useful features for catalyst and sensor polymer bases. Precursors bearing unsaturated hydroxyl and amino functionalities, as well as ferrocene, amino acids, and other moieties, were prepared using the standard procedure and proved to be good substrates for entrapment (Table 3). The xerogels were transparent or translucent glasses with a brittle fracture or, in the cases of the hydroxylated siloxanes or those containing a PEG linker, clear and semirigid glasses which showed significant fracture and tear resistance.

Although the above polymers furnished highly active encapsulates with porosities similar to the those of PGS xerogels,[29] many of them were still relatively brittle and not suited for more mechanically demanding usages. Even the inclusion of microparticulate graphite, glass microfibers, and surface treated silica did not significantly alleviate this drawback.

Recognizing that a high mesoporosity would invariably compromise the mechanical attributes of simple, single-phase sol−gel matrices, we then examined whether this problem could be resolved by forming interpenetrating polymer network

(27) Jordan, J. D.; Dunbar, R. A.; Bright, F. V. *Anal. Chem.* **1995**, *67*, 2436.

(28) The initiation, rate and extent of phase separation could be controlled by varying the sol−gel mix. Thus, macroporous methylsiloxanes and allylsiloxanes were formed at water:precursor ratios above about 0.60 and 0.35 w/w, respectively. SEM studies of the materials demonstrated three-dimensional fused globule architectures with continuous pore networks. The frameworks showed transitions from approximately 1−3 μm spherical/oblate/prolate structures in the small pore (approximately 1−15 μm) materials to 1−3 × 3−20 μm solid threadlike elements in the large pore (approximately 15−50 μm) polymers.

(29) Initial NA and TPA examinations of the metallosilicate and mesoporous alkylsiloxane sol−gels indicated respective pore distributions of 8.6−12.9 and 8.1−11.3 nm and pore volumes of 1.751−1.820 and 0.860−1.601 mL/g.

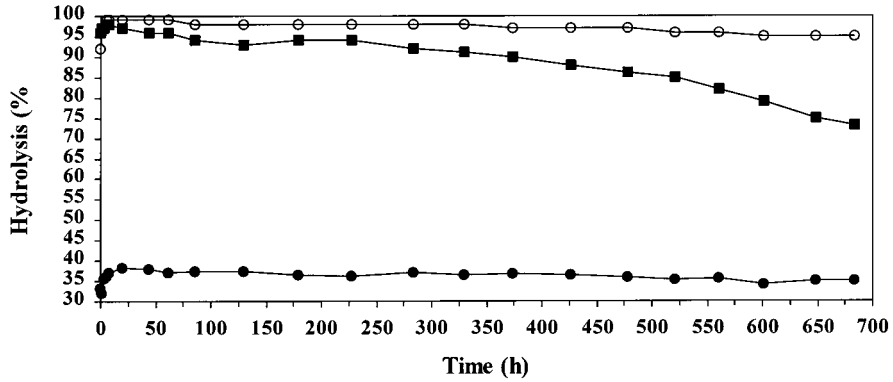

Figure 1. Continuous hydrolysis of Paraoxon by *P. dimunita* organophosphate hydrolase. Symbols: open circles, polyol ester-derived 2:1:1 PS−PDMS−silica sol−gel; filled circles, compositionally identical sol−gel prepared using PMS method; filled squares, polyurethane immobilizate. Paraoxon (25 mM) in HEPES (100 mM, pH 7.4, containing 10% v/v propan-1-ol and 0.1 mM cobalt acetate) was fed at 2.4 mL/h, 40 °C, into a glass column (0.5 × 10 cm) containing the sol−gel (0.8 g, 200−300 μm particles, loaded with enzyme at 27 mg/g or 29 mg/g) or at 1.9 mL/h through one packed with polyurethane foam (0.6 g, 200−300 μm particles, loaded with enzyme at 32 mg/g).

composites (IPNCs).[1] Thus, bio-doped IPNCs were fabricated with poly(dimethylsiloxane) (PDMS), poly(vinyl alcohol) (PVA), poly(glyceryl methacrylate) (PGMA), poly(vinylpyrrolidone) (PVP), and poly(vinylpyrrolidone-*co*-acrylate) (PVPA) (Table 3). Indeed, a range of PDMS, PGMA, PVA, and PVP sol−gel compositions gave hydrogels which underwent only minor (<6%) shrinkages during aging and drying and provided elastic or semirigid composites that were fracture and tear resistant and stable in aqueous and organic media.[30] Notably, the polyol ester precursors were readily blended with hydrophilic polymers to form materials combining the physicochemical attributes of both components. Resilient rubbers and hard, plastic-like solids were furnished, contrasting with the weak, water-swellable gels obtained with the organic polymers alone, or the brittleness of the pure xerogels. Thus, PGMA produced hard, optically transparent IPNCs (PGMA contents of 10−70% w/w), whereas PVP, PVPA, and PVA gave semirigid, transparent/translucent glassy products (polymer contents below 20−30% w/w) or highly flexible, transparent rubbers (polymer contents between about 30 and 70% w/w), and PDMS produced hard, transparent glasses (PDMS contents below about 25%) or semiflexible, translucent polymers (PDMS contents above about 25% w/w). Furthermore, the composites could be applied as adherent coatings on glass, silicon, several plastics, and ceramics using conventional masking and moulding techniques.[30,31] Mechanically robust encapsulates which can be accurately deposited as thin films and micron-scale patterns via micromoulding and microprinting technology[31] are rather desirable for sensor and diagnostic applications which require miniaturization and mass production.[3,5] Although the IPNC bioencapsulates are expected to be less porous than the pure sol−gels because of the inclusion of a second polymer phase, they still display good activities (Table 3), and this together with their superior mechanical properties makes them good candidates for more exacting usages.

Applications. Having proved the general utility of polyol silicates/siloxanes derived bioencapsulates, we then examined their actual performance in representative applications. For this purpose, they were evaluated against alkyl silicate/siloxane sol−gels and standard immobilization chemistries, taking care to ensure similar catalytic densities, catalyst geometries, and operating conditions, so as to allow a direct comparison of the immobilizates.

We first selected a simple aqueous enzymatic transformation, the continuous hydrolytic degradation of the organophosphate nerve agent Paraoxon (diethyl 4-nitrophenyl phosphate) by *P. dimunita* organophosphate hydrolase (OPH),[32] in a column reactor (Figure 1). The performance of the enzyme encapsulated in a propylsiloxane−PDMS−silica sol−gel prepared with the new approach, a chemically identical sol−gel made with PMS, and that immobilized on polyurethane foam[33] were studied. An Ormosil sol−gel was chosen so as to gain a hydrophobic matrix and thus improve substrate partitioning. As expected, the conventional encapsulate displayed a poor activity (28%), but notable stability, whereas the higher activity of the PGS sol−gel (94%), coupled with its good stability, enabled the latter to outperform the polyurethane immobilizate (initial activity 68%) in the long run. These results should be compared with the low activity reported for the enzyme entrapped in a TMOS-derived silica, when used for Parathion hydrolysis and detection.[5s]

Having established the efficiency of a polyol ester-derived bioencapsulate in an aqueous system, we then examined reactions in hydrophilic and hydrophobic solvents (Table 4), media which are of greater interest for synthetic biotransformations. For the first case, we chose the proteinase K-mediated synthesis of the pentapeptide *N*-Cbz-L-[Leu^5]-Enkephalin amide[34] and the *C. rugosa* lipase-catalyzed resolution of Ibuprofen methyl ester.[35] The enzymes were entrapped in 3-gluconamidopropylsiloxane (GAPS)−alumina−silica and propylsiloxane−poly(dimethylsiloxane)−silica, respectively, and compared with the corresponding conventional sol−gels and with cross-linked

(30) For example, such materials were obtained with PGMA and PVA at weight compositions of $(SiO_2)_{0.2-1.6}(PGMA)_{0.45-0.8}$ and $(SiO_2)_{0.3-0.6}(PVA)_{0.4-0.7}$. The materials were observed to be symmetric and microscopically homogeneous by SEM studies. Patterns were successfully printed/moulded onto glass, nylon, PEEK, silica, aluminium oxide, and alumina−ceramics, previously cleaned with 0.1 M nitric acid, etched with 0.1 M NaOH in 1:1 H_2O:MeOH, then primed with TMOS, GPTMS, or APTES. The moderate viscosity of the precursors limited the minimal dimensions of printed/molded structures down to about 30 μm.

(31) (a) Kim, E.; Xia, Y.; Whitesides, G. M. *J. Am. Chem. Soc.* **1996**, *118*, 5722. (b) Kim, E.; Xia, Y.; Whitesides, G. M. *Nature* **1995**, *376*, 581. (c) Trau, M.; Yao, N.; Kim, E.; Xia, Y.; Whitesides, G. M.; Aksay, I. A. *Nature* **1997**, *390*, 674.

(32) (a) Dumas, D. P.; Caldwell, S. R.; Wild, J. R.; Raushel, F. M. *J. Biol. Chem.* **1989**, *264*, 19659. (b) Caldwell, S. R.; Raushel, F. M. *Biotechnol. Bioeng.* **1991**, *37*, 103. (c) Dumas, D. P.; Durst, H. D.; Landis, W. G.; Raushel, F. M.; Wild, J. R. *Arch. Biochem. Biophys.* **1990**, *277*, 155.

(33) LeJeune, K. E.; Mesiano, A. J.; Bower, S. B.; Grimsley, J. K.; Wild, J. R.; Russell, A. J. *Biotechnol. Bioeng.* **1997**, *54*, 105.

(34) Gill, I.; Vulfson, E. N. *J. Chem. Soc., Perkin Trans. 1* **1992**, *6*, 667.

(35) Lalonde, J. J.; Govardhan, C.; Khalaf, N.; Martinez, A. G.; Visuri, K.; Margolin, A. L. *J. Am. Chem. Soc.* **1995**, *117*, 6845.

Table 4. Biocatalyst Applications of Sol−Gel Bioencapsulates Prepared Using Polyol Silicate/Siloxane Sol−Gel Precursors; Polyol Silicate/Siloxane-Derived Sol−Gel Bioencapsulates vs Conventional Bioimmobilizates[a]

biocatalyst	sol−gel matrix (conventional)[b]	reaction catalyzed[c]	activity (%)[d]	period (h)[e]	activity loss[f]	yield[g] start	yield[g] end
proteinase K	3:1:6 GAPS:Al_2O_3:SiO_2 *vs* (CLECs)	synthesis of *N*-Cbz-L-[Leu^5]-YGGFLNH_2 from *N*-Cbz-L-YGGOEt and L-FLNH_2*h*	91 *vs 76*	683	6 *vs 35*	66 *vs 57*	58 *vs 35*
C. rugosa lipase-1	1:7:2 PDMS:PS:SiO_2 *vs* (CRL-CLECs)	resolution of (*R,S*)-Ibuprofen methyl ester to (*S*)-Ibuprofen[i]	163 *vs 127*	226	27 *vs 16*	93 *vs 89*	70 *vs 79*
almond oxynitrilase	1:1:2 GAPS:MS:SiO_2 *vs* (AVICEL cellulose)	trans-hydrocyanation of (2*E*,4*E*)-hexa-2,4-dienal to (1*R*,2*E*,4*E*)-1-cyanohexa-2,4-diene-1-ol[j]	91 *vs 87*	322	16 *vs 53*	76 *vs 73*	68 *vs 37*
sialic acid aldolase + myokinase + pyruvate kinase + pyrophosphatase + CMP-sialate synthetase + α(2,6)sialyl transferase	2:1:7 APS:ZrO_2:SiO_2 *vs* (oxirane beads)	synthesis of α(2,6)sialyl-*N*-acetyllactosamine from *N*-acetyllactosamine and *N*-acetylmannosamine[k]	91 *vs 72* 88 *vs 54* 93 *vs 61* 89 *vs 57* 89 *vs 72* 92 *vs 64*	339	6 *vs 12* 8 *vs 23* 4 *vs 13* 9 *vs 21* 5 *vs 26* 5 *vs 20*	92 *vs 94*	85 *vs 66*
S. cerevisiae (yeast, whole cells)	3:1:4 GAPS:ZrO_2:SiO_2 *vs* (alginate beads)	reduction of ethyl 3-oxobutanoate to ethyl (3*S*)-3-hydroxybutanoate[l]	88 *vs 86*	624	8 *vs 18*	96 *vs 97*	95 *vs 83*

[a] First figure/range refers to encapsulates prepared using polyol precursors, and those in italics correspond to conventional, non-sol−gel immobilizates. [b] Xerogel composition (% w/w). The immobilizate used for comparison is indicated in brackets. [c] Reactions were conducted in continuous, packed bed reactors (enzymes) or a stirred reactor (yeast cells). [d] Initial activity of encapsulate, relative to the nonimmobilized material, in a aqueous assay. [e] Duration of reactor operation. [f] Percentage of initial activity lost during operating period. [g] Initial and final biotransformation yields. [h] 0.25 M substrates in 2:1 acetonitrile:dimethylformamide, containing 3% v/v CAPSO (0.1 M, pH 9.5), 25 °C. [i] 1.0 M substrate in 1:1:1 propanol:ethanol:triethanolamine (0.3 M, pH 7.5), 35 °C. [j] 0.2 M (2*E*,4*E*)-hexa-2,4-dienal and 0.32 M acetone cyanohydrin in diisopropyl ether saturated with acetate (0.15 M, pH 5.2), 20 °C. [k] 20 mM *N*-acetyllactosamine and 70 mM *N*-acetylmannosamine in TRIS (0.1 M, pH 8.3), 25 °C. [l] 50 mM substrate in acetate (50 mM, pH 3, containing 3.5% v/v EtOH) 25 °C.

enzyme crystals. Earlier trials with subtilisin, α-chymotrypsin, thermolysin, and papain had shown excellent performances of hydrophilic GAPS−silicate biogels in acetonitrile, DMF, DMSO, dioxane, and lower alcohols, and in accordance with previous reports,[4b,h] alkylsiloxane sol−gels were found to be the best suited to lipase entrapment. The conventional sol−gels were distinguished by low efficiencies (activities of 33% and 61%, respectively), whereas the activities of the polyol ester-derived materials were on par with those of the cross-linked crystals. Indeed, the initial activity of the *C. rugosa* encapsulate was significantly higher (163%) than that of the commercial CRL-CLECs (127%), perhaps due to greater activation of the lipase during entrapment in the siloxane sol−gel,[4b,h] together with more favorable mass transfer and substrate partitioning. Whereas the CRL-CLECs ultimately proved to be more stable than the encapsulated lipase, the reverse was observed for proteinase K, although it is possible that the use of different crystallization conditions and/or cross-linking regime could provide a more stable biocatalyst.

The oxynitrilase-mediated trans-hydrocyanation of (2*E*,4*E*)-hexa-2,4-dienal in diisopropyl ether, using acetone cyanohydrin as cyanide donor,[36] was elected for examining sol−gel performance in hydrophobic media. To sustain an aqueous-type enzyme environment, but at the same time confer some hydrophobic character upon the matrix and enhance substrate penetration, a GAPS−methylsiloxane−silica sol−gel was utilized. This proved to have good activity and stability, when compared with the standard AVICEL cellulose immobilizate, which although possessing a similar profile initially, deteriorated in the long term (Table 4).

An application where maintaining a high enzyme activity and stability is critical is the execution of reaction sequences using multi-enzyme cascades.[37] To test this aspect, we studied the synthesis of the sialyl trisaccharide α(2,6)sialyl-*N*-acetyllactosamine from *N*-acetyllactosamine and *N*-acetylmannosamine using an elegant multicatalytic cycle employing six different enzymes that has been reported in the literature.[38] A (3-aminopropyl)siloxane (APS)−silica sol−gel was selected for its hydrophilicity and its affinity for anionic species and tested against a surface-immobilized system based on oxirane−acrylate chemistry (Table 4). The activity of the polyol ester-derived encapsulate was similar to that of the oxirane−acrylate immobilizate and far superior to the standard sol−gel (activities of 28−47% for the enzymes concerned), and once again, the enhanced stability of the former was apparent during extended reactor operation (Table 4).

Having shown the synthetic utility of sol−gel enzyme encapsulates, we turned our attention to whole-cell catalysts, which are of great importance for industrial bioconversions, especially complex multistep, oxidative/reductive transformations.[39] The high sensitivity of cells limits the types of suitable encapsulants in the main to polysaccharides, which although highly biocompatible, show poor physicochemical stabilities. On the other hand, the toxic effects associated with TMOS protocols has meant that attempts at sol−gel entrapment have met with limited success.[2−5] As the polyol ester precursors had proved to be highly effective for immobilizing cells (Tables 2 and 3), we examined a model bioreduction, the yeast-mediated production of the pharmaceutical intermediate ethyl (3*S*)-3-hydroxybutyrate[40] (Table 4). A GAPS−zirconia−silica was selected as encapsulant because of its high biocompatibility and

(36) Ognyanov, V. I.; Datcheva, V. K.; Kyler, K. S. *J. Org. Chem.*, **1991**, *113*, 6992.

(37) (a) Wong, C.-H.; Whitesides, G. M. *Enzymes in Synthetic Organic Chemistry*; Tetrahedron Organic Chemistry Series, Vol. 12; Elsevier Science: Oxford, U.K., 1994. (b) Drauz, K., Waldmann, H., Eds. *Enyzme Catalysis in Organic Synthesis*; VCH Publishers: Weinheim, Germany, 1995.

(38) Ichikawa, Y.; Liu, J. L−C.; Shen, G.-J.; Wong, C.-H. *J. Am. Chem. Soc.* **1991**, *113*, 6300.

(39) (a) Davies, H. G.; Green, R. H.; Kelly, D. R.; Roberts, S. M. *Biotransformations in Preparative Organic Chemistry*; Academic Press: London, 1989. (b) Holland, H. L. *Organic Synthesis with Oxidative Enzymes*; VCH Publishers: New York, 1992.

(40) Rohner, M.; Sonnleitner, B.; Fiechter, A. *J. Biotechnol.* **1992**, *22*, 129.

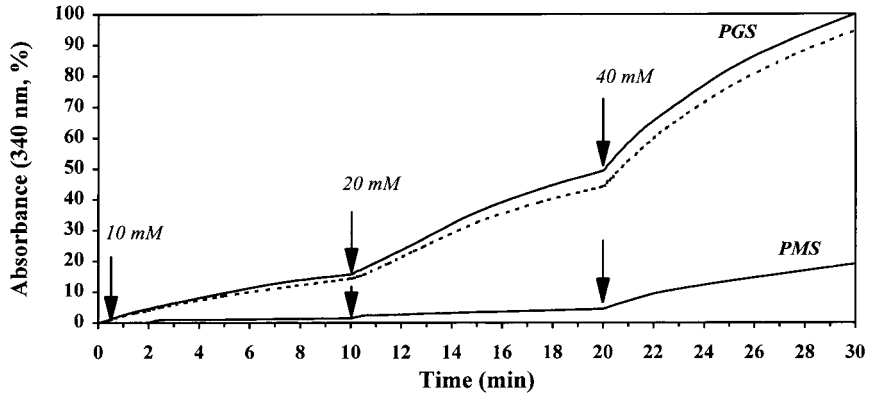

Figure 2. Performances of optical D-glucose 6-phosphate dehydrogenase sol−gel biosensors. Upper curves: PGS-derived element, with the solid and broken lines representing the fresh sensor and that after 130 h of operation respectively. Lower curve: PMS-derived sensor. A 5 × 20 × 1.7−1.8 mm strip of 1:1:2 $PVA_{85-146K}$:APS:SiO_2, doped with 14 U of Torula yeast enzyme, was placed in a stirred 1.5 mL quartz cuvette, 25 °C. The response to 1.3 mL of glucose 6-phosphate/NADP (10/15, 20/40 and 40/60 mM) in 75 mM phosphate, pH 7.4, was measured by monitoring the absorbance at 340 nm.

Table 5. Biosensor and Biodiagnostic Applications of Sol−Gel Bioencapsulates Prepared Using Polyol Silicate/Siloxane Sol−Gel Precursors; Polyol Silicate/Siloxane-Derived Sol−Gel Bioencapsulates vs Conventional Bioimmobilizates[a]

enzymes	matrix composition[b]	application	activity (%)[c]	period[d]	activity loss[e]	response time[f] start	response time[f] end
acetylcholine esterase + choline oxidase	2:3:1:4 PVA:Pd−G:GAPS:SiO_2	thick film microsensor for acetylcholine and choline[g]	91 *vs 40* 86 *vs 39*	482 h	7 *vs 5* 11 *vs 9*	10−15 s *vs 30−50 s*	12−18 s *vs 40−60 s*
glucose oxidase + galactose oxidase + lactate oxidase + horseradish peroxidase	2:1:5 PVA:HEAS:SiO_2	coated microtiter plate diagnostic for glucose, galactose, lactose and lactate[h]	92 *vs 28* 87 *vs 36* 90 *vs 26* 96 *vs 49*	60 cycles	8 *vs 3* 10 *vs 5* 9 *vs 12* 5 *vs 8*	30−50 s *vs >10 min*	35−60 s *vs >10 min*

[a] First figure/range refers to encapsulates prepared using polyol precursors, and those in italics, to the conventional sol−gels. [b] Xerogel composition (% w/w). [c] Initial activity of encapsulate, relative to the nonimmobilized material, in an aqueous assay. [d] Duration of operation. [e] Percentage of initial activity lost during testing period. [f] Response times at the beginning and end of testing period. [g] Linear operation at 0.02−2 mM acetylcholine/choline, 35 °C. [h] Linear operation at 0.5−20 mM sunstrate, 25 °C. Abbreviations: Pd−G, palladium on graphite.

physical stability and compared with alginate beads. The whole-cell activity of the GAPS sol−gel (88%) was similar to that of alginate beads (86%) and much higher than for a PMS silica (32%). Interestingly, the GAPS-biogel had a greater operational stability than the alginate encapsulate, probably due to reduced matrix degradation and cell leakage.

We next focused on an arena where high activity, fast response, good operational and storage stability, and ease of fabrication are of paramount concern, namely biosensors and biodiagnostics. Initially, we chose a previously described optical biosensor for D-glucose 6-phosphate, based on the dehydrogenase enzyme from Torula yeast.[5d] An APS−PVA−silica gel was chosen for its hydrophilicity, affinity for the substrate, and good mechanical strength and moulding characteristics. The results obtained with a polyol ester-derived sol−gel are compared with those of a chemically identical material produced using PMS, in Figure 2. The faster response of the former and its good working stability are noteworthy. Next, we examined whether this level of performance could be sustained when signal transduction and multi-enzyme systems were employed. Thus, an acetylcholine esterase−choline oxidase bi-enzymatic electromicrosensor for acetylcholine/choline and a microtiter plate optical diagnostic for sugars and lactate, based around glucose, galactose, and lactate oxidases and horseradish peroxidase, were constructed. IPN composites employing PVA−graphite−GAPS−silica and PVA−HEAS−silica chemistries were respectively employed and proved to be effective and stable sensor environments, as evidenced by their significantly better functioning over the standard PMS sol−gels (Table 5).

Thus, it appears that the most important requirements for biocatalysts and biosensors, namely high activity and stability, tolerance of different reaction media, the realization of multi-enzyme catalysis, and ease of fabrication, can be satisfied by the present encapsulates.

Conclusion

A methodology is advanced for the efficient entrapment of biologicals in sol−gel polymers ranging from glassy silicates and rubber-like ormosils to tough organic−inorganic composites. The encapsulates display activities approaching those of the native biologicals and the distinctive robustness of sol−gels and can be fabricated in various forms. The precursors are available via conventional synthetic routes, and diverse polymer chemistries can be accessed, allowing the matrix to be tailored to suit the biological and the final usage.

The underlying reasons for the high efficiencies of the PGS-derived sol−gels are not clear, although the distinctively high porosities and activities of these products as compared to PMS and PSA sol−gels, even when the latter are doped with glycerol, suggest a critical role for the microstructure. Detailed studies aimed at elucidating the mechanism of precursor hydrolysis/condensation, the initiation and evolution of the polymer/pore framework and protein−polymer interfaces, and the dependence of substrate penetration on the pore structure are required for elucidating the effectiveness of PGS sol−gels.

Although these issues and others, such as mechanical stability and porosity remain to be properly addressed, the initial results are promising and suggest applications in the biocatalyst,[3c,d,4,35,41−43] biosensor,[3e,f,5] biodiagnostic,[3e,f,6] and combinatorial biocatalysis[44] arenas. Indeed, results indicate that, at least for some proteins, the technique may provide useful

(41) Wartchow, W. A.; Wang, P.; Bednarski, M. D.; Callstrom, M. R. *J. Am. Chem. Soc.* **1992**, *114*, 378.

"off the shelf" biocatalysts for synthetic transformations,[35,41−43] as well as extended stability biosensors. The accessibility of functionalized, multicomponent, and IPN composites should also lend this technique to producing micro/macrostructure arrays with catalytic/recognition features.[3e,f,45] Finally, polyol silicates/siloxanes may prove useful for assembling cell/tissue scaffolds, as well as the synthesis of controlled architectures via biomimetic and templating routes.[46] Work is presently in progress to identify the reasons for the effectiveness of PGS sol−gels and to develop further applications of the precursors.

Experimental Section

Materials. *T. album* proteinase K, bovine α-chymotrypsin, almond *β*-glucosidase, horseradish peroxidase, rabbit muscle aldolase, *E. coli* sialic acid aldolase, *S. thermophilus* glycerol 3-phosphate oxidase, thermolysin, horse liver alcohol dehydrogenase, *C. cerevisiae* pyruvate decarboxylase, almond oxynitrilase, *C. cerevisiae* carboxypeptidese Y, *E. coli* penicillin acylase, *A. niger* glucose oxidase, soybean lipoxygenase, pig liver esterase, *S. chromofuscus* phospholipase D, subtilisin, porcine myokinase, *E. coli* pyrophosphatase, rat α(2,6)sialyl transferase, *Alcaligenes* sp. choline oxidase, rabbit pyruvate kinase, *A. globiformis* choline oxidase, bovine alkaline phosphatase, *P. pyralis* luciferase, electric eel acetylcholine esterase, *Pediococcus* sp. lactate oxidase, *D. dendroides* galactose oxidase, Porcine aspartate aminotransferase, phenolphthalein *β*-glucuronide, 4-nitrophenyl α-glucoside, 4-nitrophenyl *β*-glucoside, 4-nitrophenyl butyrate, 4-nitrophenyl phosphate, *N*-Cbz-L-phenylalanylalanine (CPA), *N*-Ac-L-phenylalanylleucinamide (APLA), *N*-Ac-L-tyrosine ethyl ester (ATEE), *N*-Bz-L-arginine-4-nitroanilide (L-BAPNA), benzylpenicillin, tyrosine, pyruvic acid, diethyl 4-nitrophenyl phosphate (Paraoxon), pyrogallol, glycerol 3-phosphate disodium salt, α-ketoglutaric acid, fructose 1,6-diphosphate, *N*-acetyllactosamine, *N*-acetylmannosamine, adenosine 5′-triphosphate, cytidine 5′-monophosphate, phosphoenol pyruvate, egg l-α-phosphatidylcholine, glycerol 3-phosphate disodium salt, glucose 6-phosphate disodium salt, NADP disodium salt, acetylcholine, choline, oxirane-acrylate beads, luciferin, 2,2′-azinobis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS), sodium alginate, poly(L-lysine) hydrobromide, (*R*,*S*)-Ibuprofen, and biological buffers were purchased from Sigma Chemical Co. Cross-linked crystals of *C. rugosa* lipase-1 (CRL-CLECS) were obtained from Altus Biologics Inc. Glycerol, TMOS, 3-aminopropyltriethoxysilane (APTES), 3-glycidoxypropyltrimethoxysilane (GPTMS), tetraprop-2-yl titanate, tetraprop-1-yl zirconate, triethyl aluminate, PVA, glycidyl methacrylate, *O*,*O*′-bis(2-aminopropyl)PEG, ethanolamine, PVP, PVPA, 2,2′-azobis(2-methylpropionamidine) dihydrochloride (ABMP), palladium(II) chloride, graphite powder, *δ*-gluconolactone, ethyl 3-oxobutanoate, dithiothreitol, ethanolamine, ferrocene carboxylic acid, ferrocene acetic acid, *N*-Cbz-L-lysine, *N*-Cbz-L-serine, DCC, acetone cyanohydrin, succinic semialdehyde, and all solvents were purchased from Aldrich Chemical Co. Bis(trimethoxysilyl)ethylene, phenyltriethoxysilane (PhTES), *N*-(2′-aminoethyl)-3-aminopropyltrimethoxysilane (AEAPTMS), 3-ureidopropyltrimethoxysilane (UPTES), silanol-terminated poly(dimethylsiloxane) (ST-PDMS), tin(II) 2-ethylhexanoate, and poly(antimony(III) ethylene glycoxide) were obtained from ABCR GmbH & Co. KG, Karlsruhe, Germany.

Analytical Techniques. HPLC was carried out on using a LDC Milton Roy CM4000 pump connected to a Spectra Physics SP8450 UV/vis detector, LDC Marathon autosampler, and an HP 35900 Chemstation, or a Waters 510 pump system connected to a Waters 717 Autosampler, an ACS light scattering detector, and a Waters data collection and integration station, or a Varian 9012 ternary pump connected to a Spectra Physics RI detector and a Spectra Physics SP4290 Integrator module. Analyses were performed using a Hichrom RPB5 column (5 μm, 0.46 × 15 cm) eluted with water−acetonitrile or water−methanol, 20−40 °C, a Hypercarb column (10 μm, 0.46 × 10 cm) eluted with water−acetonitrile, 20−40 °C, a Bio-Rad Aminex HPX-87C column (10 μm, 0.46 × 25 cm) eluted with 0.5−5 mM calcium nitrate, 50−70 °C, or a Chiracel OJ column (5 μm, 0.46 × 25 cm) eluted with hexane−propan-2-ol, 30 °C. GC was performed on a Varian Star 3400CX capillary GC equipped with an autosampler and FID detector and connected to a Varian Star data acquisition and analysis station. Analyses were performed on a 30 m SPB-1 column (0.25 mm diameter, 0.25 μm film thickness) or SPB-5 column (0.50 mm diameter, 0.5 μm film thickness) with helium as carrier gas. Analyses were typically carried out using an injection temperature of 200−250 °C, a split ratio of 25−100, a total flow of 20−40 mL/min, a detector temperature of 250 °C, and a ramp of 50−250 or 75−300 °C over 20−30 min. UV−vis spectrometry was carried out on a Kontron 930 spectrometer equipped with a thermostated, stirred cell, a Perkin-Elmer Lambda-2 spectrometer equipped with a thermostated, stirred flow cell, or a Shimadzu UV-1602 spectrometer. NMR spectra were recorded on a JEOL EX270 FT spectrometer: ^{1}H and ^{13}C were recorded at 270 and 67.8 MHz, respectively, using a 5 mm probe, and ^{29}Si were recorded at 39.76 MHz using a 10 mm probe. TMS was used as internal standard in all cases. FAB-MS was carried out on a Kratos MS9/50TC spectrometer using a xenon gun operated at 5−7 kV or on a VG AUTOSPEC spectrometer using a cesium gun operated at 30 kV. The samples were applied to a polished copper probe, either neat or dispersed in thioglycerol. Spectra were recorded in positive ion mode, using polyglycerol or poly(thioglycerol) ions as the reference. Nitrogen adsorption (NA) isotherms were determined using a Micrometrics ASAP-2000 instrument. Specific surface areas were calculated by five-point BET analysis, and the Kevin function was used to obtain the pore distribution. The xerogels were washed for 20 h at room temperature with distilled water, then methanol, and finally with pentane, dried under nitrogen at room temperature for 20 h, then degassed at 0.02 mbar, 20 °C for 60 h, before analysis. Thermoporometric (TPM) analysis was implemented using a Perkin-Elmer DSC-7 Instrument interfaced with a TAC-7/7 Instrument Controller, PE DSC-7 cooling units, and PETA-DSC-7 control software. Hydrogel and xerogel samples were washed with 2:1 water:methanol for 72 h, then with distilled water for 72 h, then vacuum infiltrated and soaked in distilled water for 1 week prior to analysis. Measurements were performed over the range −50 to 20 °C, with heating and cooling rates of 0.5 or 1 °C/min. Pore size, volume and shape, and surface area were calculated from the corresponding freezing temperatures using Laplace and Gibbs−Duhem functions, the observed enthalpy of freezing/melting, and the shape factors determined from melting−solidification hysteresis experiments.[19] SEM was carried out on a Hitachi S-570 SEM Unit, using Edwards S150 and ISI DS-130 sputter gold coaters.

Preparation of Methyl/Ethyl Ester and Polyol Ester Precursors. (i) Poly(methyl silicate) (PMS) and poly(glyceryl silicate) (PGS): TEOS (0.48 mol) was mixed with ethanol (50 mL), and hydrochloric acid (10.4 mL of 0.25 M) was added over 30 min with vigorous stirring; then the mixture was heated to 70 °C for 15 h. Rotary evaporation at 35 °C provided PMS of composition $SiO_{1.1-1.2}(OMe)_{1.6-1.8}$ as a clear, viscous liquid. PGS was obtained by adding glycerol (0.38 mol) to the reaction mixture over 1 h, heating to 50 °C, and stirring for a further 40 h. Concentration at 50 °C to about 70% w/w solids, and further evaporation at 20 °C gave a gel of composition $SiO_{1.16-1.19}Glc_{0.8}(OEt)_{0.02-0.08}$. Poly(antimony(III) ethylene glycoxide) catalyst gave similar results. FAB-MS indicated that the product consisted mostly of glyceryl-bridged linear oligomeric polysilicates of DP 5−9. Various

(42) (a) Khalaf, N.; Govardhan, C. P.; Lalonde, J. J.; Persichetti, R. A.; Wang, Y.-F.; Margolin, A. L. *J. Am. Chem. Soc.* **1996**, *118*, 5494. (b) Wang, Y.-F.; Yakovlevsky, K.; Zhang, B.; Margolin, A. L. *J. Org. Chem.* **1997**, *62*, 3488.

(43) (a) Wang, P.; Sergeeva, M. V.; Lim, L.; Dordick, J. S. *Nat. Biotechnol.* **1997**, *15*, 789. (b) Panza, J. L.; LeJeune, K. E.; Venkatsubramanian, S.; Russell, A. J. *ACS Symp. Ser.* **1998**, *680*, 134. (c) LeJeune, K. E.; Panza, J. L.; Venkatsubramanian, S.; Russell, A. J. *Polym. Prepr.* **1997**, *38*, 563.

(44) Mozhaev, V. V.; Budde, C. L.; Rich, J. O.; Usyatinsky, A. Y.; Michels, P. C.; Khmelnitsky, Y. L.; Clark, D. S.; Dordick, J. S. *Tetrahedron* **1998**, *54*, 3971.

(45) Kim, E.; Xia, Y.; Whitesides, G. M. *J. Am. Chem. Soc.* **1996**, *118*, 5722.

(46) (a) Davis, S. A.; Burkett, S. L.; Mendelson, N. H.; Mann, S. *Nature* **1997**, *385*, 429. (b) Ozin, G. A. *Acc. Chem. Res.* **1997**, *30*, 17. (c) Mann, S.; Burkett, S. L.; Davis, S. A.; Fowler, C. E.; Mendelson, N. H.; Sims, S. D.; Walsh, D.; Whilton, N. T. *Chem. Mater.* **1997**, *9*, 2300. (d) Tanev, P. T.; Liang, Y.; Pinnavaia, T. J. *J. Am. Chem. Soc.* **1997**, *119*, 8616. (e) Raman, N. K.; Anderson, M. T.; Brinker, C. J. *Chem. Mater.* **1996**, *8*, 1682.

glyceryl silicates ("$SiGlc_{2-4}$") and poly(glyceryl silicates) ("$SiO_{0.5-1.5}Glc_{0.5-2}$") were prepared by this method. (ii) Poly(alkoxyalkylsiloxanes) "$RSiO_{0.5}(OAlk)_2$", poly(glyceroxyalkylsiloxanes) "$RSiO_{0.5}Glc$", poly(alkyl metallosilicates) "$SiM_{0.1-0.2}O_{1.1-1.2}(OAlk)_{2.1-2.6}$", and poly(glyceryl metallosilicates) "$SiM_{0.1-0.2}O_{1.1-1.2}Glc_{1.9-2}$": The same method was used as in (i), except that the poly(alkylsiloxanes) utilized alkyltrimethoxysilanes and alkyltriethoxysilanes as substrates, and with the metallosilicates, condensation was conducted using TEOS and 10−20 mol % of tetraprop-2-yl titanate(IV), tetrapropyl zirconate(IV), or triethyl aluminate(III) as the metal species. With poly(3-aminopropylsiloxane), APTES was used without any catalyst. The polyol products contained 2−8 mol % of ethoxide, and FAB-MS indicated bridged oligomers with DPs of 4−11. (iii) 3-Gluconamidopropylsiloxane "3-GAPSi": APTES (0.28 mol in 20 mL of methanol) was added over 1 h to a stirred suspension of δ-gluconolactone (0.28 mol in 100 mL of methanol) heated to 50 °C, 40 h, then the mixture evaporated at 40 °C to give a pale yellow solid containing 4 mol % of methoxide. (iv) PMS and PGS with 10 mol % of 3-(1′-hydroxy-2′-(2″-hydroxyethylamino)ethoxy)propylsiloxane "$(HEPSiO(OMe))_{0.1}(SiO(OMe)_2)_{0.9}$" and "$(HEPSiOGlc)_{0.1}(SiOGlc)_{0.9}$", or 10 mol % of *O*,*O*′-bis(2-(2′-hydroxy-2′-(3″-oxysilylpropoxy)ethylamino)propyl)-PEG_{500} "$(BHP(SiOOMe)_2)_{0.1}(SiO(OMe)_2)_{0.9}$" and "$(BHP(SiOGlc)_2)_{0.1}(SiOGlc)_{0.9}$": GPTMS (0.1 mol) was added over 5 h to stirred ethanolamine (0.1 mol) or *O*,*O*′-bis(2-aminopropyl)PEG_{500} (50 mmol) heated to 50 °C. After dilution with methanol (25 mL) and stirring for 20 h, TMOS (0.9 mol) was added, followed by water (1.09 mol, over 1 h). Further heating for 20 h, followed by evaporation at 50 °C gave the PMS-HEAS/BHP products as a viscous yellow liquids. The addition of glycerol (0.81 or 0.86 mol, over 1 h), with further heating and stirring for 40 h, followed by concentration at 50 °C to about 70% w/w solids, and evaporation at 20 °C, provided the GPS-HEAS/BHP products as yellow gels retaining 3−8 mol % of methoxide. (v) PMS and PGS with 45 mol % of PDMS "$PDMS_{0.45}(SiO(OMe)_2)_{0.55}$" and "$PDMS_{0.45}(SiOGlc)_{0.55}$": ST-PDMS (0.27 mol equiv of Me_2SiO) was added over 5 h to TMOS (0.33 mol) containing 1% w/w of tin(II) 2-ethylhexanoate, heated to 50 °C. After 30 h, hydrochloric acid (5.94 mL of 0.25 M) was added over 2 h, stirring continued for 30 h, and the solution evaporated at 50 °C to give the PMS-PDMS product as a clear viscous liquid. The addition of glycerol (30 g, 0.33 mol in 15 mL of ethanol) over 0.5 h, followed by stirring for 40 h, then concentration at 50 °C to approximately 60% w/w solids, and continued evaporation at 20 °C, provided the GPS-PDMS material as an opalescent gel containing 5−7 mol % of methoxide. (vi) Methoxy and glyceroxy *N*-acyl-3-aminopropylsiloxanes bearing amino acid or ferrocene moieties "$XPSi(OMe)_3$" and "$XPSiGlc_3$": A solution of DCC (11 or 33 mmol) in dichloromethane was added to a solution of APTES (10 or 30 mmol) and *N*-Cbz-amino acid, ferrocene acetic acid or ferrocene carboxylic acid (10 or 30 mmol) in 1:1 dichloromethane:acetonitrile, and the mixture stirred at room temperature for 4−20 h. A few drops of acetic acid and methanol were added, the mixture stirred for 2 h, then filtered. The *N*-(N_α-Cbz-aminoacyl)-APTES compounds were deprotected by hydrogenation in methanol, using palladium−carbon or palladium−alumina as catalyst. The *N*-ferrocenyl-APTES and *N*-aminoacyl-APTES derivatives were reacted with three equivalents of glycerol as in (i) to give the triglyceroxysiloxanes as viscous liquids or gels, retaining 3−7% of methoxide.

NMR Studies of Alkyl Silicate and Polyol Silicate Hydrolysis. The rates of hydrolysis of polyol silicates, poly(polyol silicates), TMOS, and poly(dimethyl silicate) and their condensation and cross-linking profiles were determined by following the appearance of the characteristic ^{29}Si-OH signal between −73 and −84 ppm in solutions containing 20−200 mM of precursor, typically dissolved in 1:8 or 1:4 methanol-d_4:50 mM phosphate, pH 7, containing 10 mM TRIS(acetylacetonato)chromium(III) as ^{29}Si relaxant, 5 °C.[17]

Encapsulation of Biologicals. (i) Silica, metallosilicates, alkylsiloxanes, and functionalized siloxanes: (a) PMS and conventional precursors: In a typical procedure, ice-cold precursor mix (1 g) was stirred with methanol (0−0.2 mL) and ice-cold buffer (0.3−2 mL). Aqueous ammonium fluoride (0.5 M) was then added to a concentration of 15−50 mM, followed by a solution/suspension of the biological (0.2−2.0 mL) after 0−3 min, and the mixture left stirring over ice to gel (0.3−3 min for silicates, metallosilicates, and composites; 2−10 min for functionalized siloxanes; 5−20 min for min for alkylsiloxanes). The overall methanol contents (cosolvent plus that released upon hydrolysis) of the sol−gels varied between 23% and 40% v/v. The hydrogel was aged at 5 °C for 10−20 h in a closed container, then ground into a fine powder. The cell-containing gels were washed with phosphate (3 × 50-fold volumes of 50 mM, pH 7, 2 h), then transferred to a solution consisting of 5% potassium chloride in phosphate (50 mM, pH 7), or freeze-dried, and kept at 5 °C until used. The protein-containing gels were dried at 5 °C or room temperature (RT) for 1−2 weeks in a PTFE container provided with 0.5 mm holes, washed with phosphate (3 × 50-fold volumes of 50 mM, pH 7−8.5, containing 5 mM of calcium and magnesium acetates, 2 h), then air-dried at 5 °C or RT, or freeze-dried, and stored at 5 or −15 °C. (b) GPS and glyceryl ester precursors: The precursor mix (1.0 g) was dispersed in ice-cold water (0.2−1 mL), the biological (in 0.3−2 mL of ice-cold buffer), and the mixture left on ice to gel (0.1−5 min for silicates, metallosilicates, and composites; 3−15 min for functionalized siloxanes; 2−40 min for alkylsiloxanes). The hydrogel was aged at 5 °C for 10−40 h, then ground into a fine powder and air-dried at 5 °C or RT for 5−20 h. The protein-containing materials were washed as in (a), then air-dried at 5 °C or RT, or freeze-dried, and stored at 5 or −15 °C. The cell-containing encapsulates were treated as in (a). (ii) PDMS-silicate: Same method as for the silica/metallosilicate matrices. (iii) Alginate-silicate: A mixture of PMS/PGS and 4% w/w alginate was used for immobilization, followed by soaking in 5% w/w calcium acetate, then processed as above. (iv) PGMA-silicate: The mix contained GMA and 0.75% w/w of ABMP. Polymerization was conducted under nitrogen at 15 °C, 2−3 d. Further processing was as above. (v) PVA/PVP/PVPA-silicate: PMS/PGS together with a 10−15% w/w PVA/PVP/PVPA solution was employed for immobilization. Further processing was as above.

Bioencapsulate Evaluation. The freshly prepared and powdered bioencapsulates were sieved to recover 50−100, 100−200, and 200−300 μm fractions, washed with TAPS, TAPSO, HEPES, or phosphate (3 × 20-fold volumes of 50−100 mM, pH 7−8.5, 2 h) to remove remaining precursors, nonentrapped biological, etc. The amount of biological leached out was determined by the Lowry method, or OD measurements and microscopy (cells). Further processing was as above. The initial activities and those following dry or wet storage at 5 °C for 10 months were compared with those of the nonimmobilized biologicals using standard assays. The encapsulation conditions and assays are described in the Supporting Information.

Encapsulation of Trypsin in Silica Sol−Gels. Trypsin was encapsulated using PGS, PMS, PMS plus glycerol, PSA, and PSA plus glycerol, at Si:H_2O (mole) ratios of 3 and 9. PSA was prepared according to the reported method,[4l] except that a starting Si:ethanol:H_2O ratio of 1:4:10 was utilized, and hydrolysis was carried out at 50 °C. Exhaustive evaporation of the ethanol at 30 °C gave a PSA solution with a solids content of 35−38% w/w, which was stable at RT for 1−2 days. Immobilization with PGS and PMS was implemented according to the general procedure (above), using a solution of trypsin (2−30 mg/mL) in phosphate (100 mM, pH 7). In the case of PSA, no catalyst was employed. It was not possible to use the PSA method at a Si:H_2O ratio of 1:3, due to premature gelation of the concentrated PSA stock. As required for the PMS and PSA protocols, a quantity of glycerol identical to that generated in the PGS method (i.e., 26% w/w and 43% w/w at Si:H_2O ratios of 1:9 and 1:3, respectively) was included in the sol−gel solution prior to addition of protein. The biogels were processed as above.

Kinetic Properties of Encapsulated Trypsin and Alkaline Phosphatase. Trypsin was entrapped in pure silica using PMS, PSA, or PGS at an H_2O:Si molar ratio of 6. Xerogels with loadings of 7.8−8.2 mg/g were prepared using enzyme (2 mg/mL) in TRIS (100 mM, pH 8, containing 15 mM calcium acetate and 5mM magnesium acetate). Kinetic parameters were obtained from spectrophotometric assays performed with the free enzyme (0.1 mg/mL) or encapsulate (20 mg/mL of 100−200 μm particles), using L-BAPNA (0.2−20 mM) as substrate in TRIS (50 mM, pH 7.6, containing 15 mM calcium acetate, 5 mM magnesium acetate, and 10% v/v methanol), 20 °C. Trypsin inhibition by poly-L-lysines was determined by a competition assay

using L-BAPNA (2 mM) and inhibitor (15 mg/mL), 20 °C. Alkaline phosphatase was similarly entrapped using PMS or PGS at an H_2O:Si ratio of 6. Xerogels with loadings of 4.0−4.1 mg/g were prepared using a enzyme (1 mg/mL) in glycinate (100 mM, pH 8, containing 5 mM calcium acetate, 1 mM magnesium acetate, and 1 mM zinc acetate). Kinetic parameters were obtained from the hydrolysis of NPP (0.01−20 mM) by the free enzyme (0.05−0.1 mg/mL) or immobilizate (5−25 mg/mL of 100−200 μm particles), in glycinate (100 mM, pH 10, containing 5 mM calcium acetate, 1 mM zinc acetate, 1 mM magnesium acetate, and 5% v/v of methanol), 35 °C.

Thermostabilities of Encapsulated *C. rugosa* Lipase, β-Glucosidase, and Acid Phosphatase. The enzymes were entrapped in PMS- or PGS-derived silica at an H_2O:Si molar ratio of 6. Xerogels with loadings of 8.6−9.8 mg/g were prepared using enzyme (10 mg/mL) in TRIS (100 mM, pH 7.5, containing 25 mM calcium acetate and 10 mM magnesium acetate). Thermostabilities were determined by incubating free enzyme (1 mg/mL) or immobilizate (1 mg/mL equivalent of 100−200 μm particles) in TRIS (100 mM, pH 7, containing 25 mM calcium acetate and 10 mM magnesium acetate), at 70 °C. The residual activities of *C. rugosa* lipase and β-glucosidase were determined as already described (see general procedure). Acid phosphatase activity was determined from the hydrolysis of NPP (10 mM) in acetate (50 mM, pH 4.8, containing 10% v/v of methanol), 35 °C.

Representative Applications. Phosphotriesterase-Catalyzed Hydrolysis of Paraoxon. Paraoxon (25 mM) in HEPES (100 mM, pH 7.4, containing 10% v/v propan-1-ol and 0.1 mM cobalt acetate) was pumped at 2.4 mL/h into a glass column (0.5 × 10 cm) containing 2:1:1 PS−PDMS−silica sol−gel (0.8 g of 200−300 μm particles, loaded with *P. dimunita* enzyme at 27 mg/g), at 2.4 mL/h into one holding a conventional 2:1:1 PS−PDMS−silica gel (0.8 g of 200−300 μm particles, loaded with enzyme at 25 mg/g), or at 1.9 mL/h through one packed with polyurethane foam (0.6 g of of 200−300 μm particles, loaded with enzyme at 32 mg/g). The columns were maintained at 40 °C, and reactions were followed by UV−vis spectrometry. The polyol sol−gel, standard sol−gel, and polyurethane immobilizates showed immobilizations of 86%, 79%, and 87%, and relative specific activities of 94%, 28%, and 68%, respectively. Their preparation is detailed in the Supporting Information.

Proteinase K-Mediated Synthesis of *N*-Cbz-L-[Leu^5]-Enkephalin Amide. A solution of *N*-Cbz-L-tyrosylglycylglycine ethyl ester (0.25 M) and L-phenylalanylleucinamide (0.25 M) in 2:1 acetonitrile:dimethylformamide containing 3% v/v of CAPSO (100 mM, pH 9.5, containing 10 mM calcium acetate) was fed at 1.2 mL/h through a glass column (0.5 × 10 cm) packed with a 3:1:6 GAPS−alumina−silica polyol sol−gel (1 g of 50−200 μm particles, loaded with enzyme at 37 mg/g), at 1.2 mL/h into one holding a conventional 1:6 alumina−silica sol−gel (1 g of 50−200 μm particles, loaded with enzyme at 39 mg/g), or at 3 mL/h into one packed with cross-linked crystals (100 mg, sandwiched between 0.5 cm layers of 50−100 μm silica). The columns were maintained at 25 °C, and reactions were followed by RP-HPLC (Hichrom RPB5 column eluted with water-methanol, 40 °C, with UV−vis detection). The polyol sol−gel, standard sol−gel, and cross-linked crystal catalysts gave immobilizations of 87%, 93%, and 55−60%, and relative specific activities of 91%, 33%, and 54−81%, respectively. Their preparation is described in the Supporting Information.

***C. rugosa* Lipase-Catalyzed Resolution of (*R,S*)-Ibuprofen Methyl Ester.** A solution of (*R,S*)-Ibuprofen methyl ester (1 M) in 1:1:1 propanol:ethanol:TEA (0.3 M, pH 7.5, containing 20 mM calcium chloride) was pumped at 0.6 mL/h through a glass column (1 × 10 cm) holding a 1:7:2 PDMS−PS−silica polyol sol−gel (2.7 g of 50−100 μm particles, loaded with enzyme at 9.6 mg/g), at 0.6 mL/h into one packed with a standard 1:7:2 PDMS−PS−silica sol−gel (2.9 g of 50−100 μm particles, loaded with enzyme at 9.2 mg/g), or at 0.7 mL/h through one holding CRL-CLECS (25 mg, packed between 0.5 cm layers of 50 μm silica). The columns were maintained at 35 °C. Reactions were followed by chiral HPLC, and (*S*)-Ibuprofen ee's were found to be 91−96%. The polyol and standard sol−gel ommobilizates showed immobilization of 93% and 87%, and relative specific activities of 163%, 61%. Their preparation is detailed in the Supporting Information.

Oxynitrilase-Mediated Trans-Hydrocyanation of (2*E*,4*E*)-Hexa-2,4-dienal. A solution of (2*E*,4*E*)-hexa-2,4-dienal (0.2 M) and acetone cyanohydrin (0.32 M) in diisopropyl ether saturated with acetate (0.15 M, pH 5.2) was fed at 4.2 mL/h into a glass column (1.5 × 15 cm) packed with of 1:1:2 GAPS−MS−silica sol−gel (3.1 g of 100−200 μm particles, loaded with enzyme at 13.7 mg/g), at 4 mL/h into one holding a standard 1:2 MS−silica sol−gel (3 g of 100−200 μm particles, loaded with enzyme at 12.8 mg/g), or at 4.1 mL/h into one containing AVICEL (4.0 g, loaded with enzyme at 11 mg/g). Both columns were maintained at 20 °C, and reactions were followed by chiral HPLC. The product ee's ranged over 95−97%. The polyol sol−gel, conventional sol−gel, and AVICEL biocatalysts showed immobilizations of 89%, 85%, and 74% and relative specific activities of of 91%, 58%, and 87%, respectively. Their preparation is described in the Supporting Information.

Multi-enzymatic Synthesis of α(2,6)Sialyl-*N*-acetyllactosamine. A deoxygenated solution of *N*-acetylmannosamine (70 mM), *N*-acetyllactosamine (20 mM), cytidine 5′-monophosphate (1.5 mM), adenosine 5′-triphosphate (0.15 mM), phosphoenol pyruvate (75 mM), and pyruvate (100 mM) in TRIS (100 mM, pH 8.3, containing 5 mM dithiothreitol, 20 mM magnesium chloride, 5 mM manganese(II) acetate, and 20 mM potassium chloride) was used as the feed. This was fed at 0.15 mL/h through a glass column (0.5 × 10 cm) containing 2:1:7 APS−zirconia−silica sol−gel (1.3 g of 200−300 μm particles, doped with sialic acid aldolase (SAA) at 183 U/g, pyrophosphatase (PP) at 48 U/g, CMP−sialic acid synthetase (SAS) at 1.1 U/g, myokinase (MK) at 462 U/g, pyruvate kinase (PK) at 632 U/g, and α(2,6)sialyltransferase (ST) at 0.38 U/g). Also, the mixture was fed at 0.15 mL/h through a column holding a standard 2:8 APS−silica sol−gel (1.3 g of 200−300 μm particles, loaded with SAA at 186 U/g, PP at 46 U/g, SAS at 1.1 U/g, MK at 478 U/g, PK at 639 U/g, and ST at 0.39 U/g) or at 1.4 mL/h through one packed with oxirane−acrylate beads (1.8 g of 230−270 μm, macroporous, loaded with SAA at 170 U/g, PP at 42 U/g, SAS at 1.2 U/g, MK at 413 U/g, PK at 618 U/g, and ST at 0.36 U/g). The columns were maintained at 25 °C, and reactions were followed by HPLC. The polyol sol−gel showed a net encapsulation of 91% and relative activities of 91%, 88%, 93%, 89%, 89%, and 92%, the conventional sol−gel 93% immobilization and relative activities of 36%, 47%, 44%, 28%, 39%, and 41%, and the oxirane−acrylate immobilizate 93% immobilization and relative activities of 72%, 54%, 61%, 57%, 72%, and 64%. Their preparation is detailed in the Supporting Information.

Yeast-Mediated Preparation of Ethyl (3*S*)-3-Hydroxybutanoate. A solution of ethyl 3-oxobutanoate (50 mM) in acetate (50 mM, pH 3.0, containing 10 mM calcium acetate, 3.5% w/w of ethanol, and 0.1% v/v Silcolapse) was supplied at 3.3 mL/h into a stirred reactor (50 mL) sparged with oxygen (30%) and holding a 3:1:4 GAPS−zirconia−silica sol−gel (7.4 g of 0.7−1.5 mm particles, loaded with cells at approximately 300 mg/g), at 3.3 mL/h into one containing a conventional 1:4 zirconia-silica sol−gel (14 g of 0.7−1.5 mm particles, loaded with cells at approximately 150 mg/g), or at 3.7 mL/h into one holding alginate beads (9.6 g of 0.8−1.3 mm beads, loaded with cells at approximately 250 mg/g). The reactors were maintained at 25 °C, and reactions were followed by HPLC. The alginate beads, polyol sol−gel and standard sol−gel gave encapsulations of 98%, 93%, and 85% and relative activities of 86%, 88%, and 32%, respectively. Their preparation is described in the Supporting Information.

Optical Thick Film Biosensor for D-Glucose 6-Phosphate. Composite sol−gel strips (1.7−1.8 × 5 × 20 mm) of composition 1:1:2 $PVA_{85-146K}$:APS:SiO_2 and doped with D-glucose 6-phosphate dehydrogenase were made using the polyol ester and standard approaches. They were doped with Torula yeast D-glucose 6-phosphate dehydrogenase at approximately 87 and 81 U/g, respectively. The sensor strips were conditioned by immersion in phosphate (75 mM, pH 7.4, containing 10 mg/mL NADP, 30 min), then placed in a 0.5 × 1 × 3 cm quartz cuvette equipped with stirring. The response to 1.3 mL of glucose 6-phosphate/NADP (5−50 and 7.5−75 mM, respectively, in 75 mM phosphate, pH 7.4) was then measured by monitoring the

absorbance at 340 nm, 25 °C. Sensor stability was examined by placing the strip in a 3 mL flow cell, passing through substrate solution (5 mM substrate and 7.5 mM NADP) at 5 mL/h, 25 °C, and examining the response at selected time intervals. The polyol-derived and standard strips showed encapsulations of 89% and 83% and relative specific activities of 87% and 31%, respectively. Their preparation is detailed in the Supporting Information.

Thick Film Electrobiosensor for Acetylcholine and Choline. Polyol-derived and standard composite 2:3:1:4 PVA:Pd−C:GAPS:silica sol−gel sensors (1 × 10 mm, 200 μm thick) loaded with acetylcholine esterase and choline oxidase were printed onto glass slides. The strips were respectively loaded with approximately 0.75 and 0.66 kU/g of acetylcholine esterase and 1.12 and 1.0 kU/g of choline oxidase. The sensors were connected via 0.25 mm Teflon-sheathed copper leads (attached using silver-loaded epoxy resin) to saturated calomel and platinum microelectrodes, fixed on either side of the strips (with nonconductive epoxy resin). Measurements were conducted by placing the slides onto a thermostated aluminum block maintained at 35 °C, placing a drop of analyte solution (20−50 μL) containing acetylcholine/choline (0.02−10 mM) in oxygen-saturated phosphate (50 mM, pH 8) onto the sensor zone, and monitoring the resulting current at an overpotential of 0.2−0.9 V, using a EG&G PARC 273 analyzer. For the polyol-derived sensors, linear responses were observed over the range 0.02−2 mM for acetylcholine and 0.02−3 mM for choline, and reactions were complete within 2−5 min. For the standard sensor responses were linear for 0.02−1 mM acetylcholine/choline, and reactions were over in 10−15 min. For continuous assessment, a sensor element with all electrical contacts sealed with silicone was placed in a thermostated flow cell (2 mL) and exposed to analyte solution (0.2 mM) at an OP of 0.7 V, 35 °C. The sensor was withdrawn at selected time periods and washed thoroughly with buffer, and its response to analyte (0.02, 0.2 and 2 mM) at an OP of 0.6−0.8 V determined. The polyol-derived and standard sol−gel strips showed net encapsulations of 86% and 81% and relative specific activities of 91% and 86% and 41% and 39%, respectively. Their preparation is described in the Supporting Information.

Microtiter Plate Diagnostic for Glucose, Galactose, Lactose, and Lactate. The wells of 96-well polycarbonate microtiter plates were coated with polyol-derived 2:1:5 PVA:HEAS:silica (approximately 700 μm films) and standard 3:5 PVA:silica sol−gels doped with glucose oxidase, galactose oxidase, lactate oxidase, and horseradish peroxidase. The plates were respectively loaded with approximately 0.35 and 0.37 U/well of the oxidases and 1.17 and 1.22 U/well of horseradish peroxidase. The responses of the plates to glucose, galactose, lactose, or lactate solutions (100 μL of 0.5−50 mM) in oxygen-saturated phosphate (50 mM, pH 6, containing 0.2−70 mM ABTS, 5 mM each of calcium and magnesium acetates) were monitored at 415 nm, 25 °C, using a Molecular Devices Microplate Reader. For the polyol-derived sol−gels, reactions were complete within 2−12 min, and a linear response was observed over the ranges 0.5−20, 0.5−30, 0.5−25, and 0.5−40 mM for glucose, galactose, lactose, and lactate, respectively. For the standard sol−gels the linearity was similar, but reactions were much slower, being over in 15−130 min. For the cycling experiments, the wells were washed with phosphate (5 × 100 μL of 50 mM, pH 6, containing 5 mM each of calcium and magnesium acetates, 15 min) between analyses. The polyol-derived and standard biogels showed net encapsulations of 88% and 92%, and relative specific activities of 92%, 87%, 90%, and 96% and 28%, 36%, 26%, and 49%, respectively. Their preparation is detailed in the Supporting Information.

Acknowledgment. I.G. gratefully acknowledges the provision of a Postdoctoral Fellowship (No. BIO2-CT93-5542) by the EU under the BRIDGE program. We are indebted to Dr. J. M. Palacios and Mrs. L. Bajón at the IC-CSIC, Madrid, Spain, for SEM analyses of the sol−gel materials and to Drs. B. Brooker and R. Stenning for the use of the SEM facilities at IFR, Reading, U.K. Also, the comments and assistance of Drs. G. Bretler, U. Jenelten, R. Valivety, A. Milqvist-Fureby, A. Richards, and F. Plou during manuscript preparation are appreciated.

Supporting Information Available: Experimental details (16 pages, print/PDF). See any current masthead page for ordering information and Web access instructions.

JA9814568