Bioactive Sol-Gel Coatings

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Received March 30th, 2000

Keywords: Enzyme catalysis, Immobilization, Organic-inorganic hybrid composites, Thin films, Sol-Gel coatings

Abstract. Recent sol-gel techniques enable bioactive composite layers to be prepared by the embedding of bioactive compounds, biomolecules (BMs) and cellular systems within inorganic layers. These novel bioactive layers offer interesting new applications, *e.g.* biocompatible coatings on implants and medical products, the preparation of biosensors and biocatalysts, and coatings that can release biocides in a controlled manner.

Contents

Combining modern materials with biological science is currently one of the most innovative of technology drivers [1]. Nature itself contains examples of so-called bioengineered materials, mostly in the form of inorganic-organic composites such as bone, teeth, diatoms and seashells fabricated through highly coupled synthesis and assembly. These structures are formed at room temperature in aqueous systems by means of template-assisted self-assembly in which organic materials (*e.g.* proteins, lipids or both) of the order of length of 1 to 100 nm form the structural scaffolding for the deposition of specifically oriented and shaped inorganic materials such as hydroxyapatite, $CaCO_3$ or SiO_2 [2, 3]. The special architecture of such nanocomposite materials, especially the large number of internal interfaces, results in for example outstanding mechanical properties, since they are simultaneously hard, strong and tough.

Great efforts have been made to understand the common principles underlying the formation of such materials in order to be able to either generate new materials or modify existing ones for a wide range of material science applications, including the biomaterials field. Based on these biological principles (”biomimetics” [2]), bio-inspired systems and materials are now being formed by self-assembly or other patterning methods to provide new routes to advanced and ”smart” materials for a wide range of applications in the chemical, pharmaceutical and electronics industries, *e.g.* materials for bone and tissue regeneration, drug-delivery systems, and new forms of organized and nanostructured systems.

A current trend in the field of bio-engineered materials is the investigation of nanocomposites in the form of bulk products and thin layers, where bioactive compounds are embedded within inorganic nanostructured oxide matrices. The preparation of such bioactive composites has been simplified by developments in sol-gel techniques [4–10] over the last 10 years.

This report summarizes the preparation, properties and prospective applications of bioactive sol-gel coatings.

1. Preparation of Bioactive Sol-Gel Coatings

Thin coatings are of increasing importance for several reasons when modifying and refining material surfaces. Thin-film based materials require only small amounts of embedded functional molecules, exhibit fast response times with external reagents, are compatible with optics and electronics, have great potential for miniaturisation, and also permit the possibility of preparing multi-layer configurations.

Thin bioactive layers are increasingly of interest as they can either accelerate or slow down the rate of biological, biochemical or biotechnological processes. This biofunctionality can be realized by a number of different means:

1) The structure and composition of the layer surface, *e.g.* one that promotes biomineralization processes (biocompatible surfaces),
2) Immobilization of biocomponents such as enzymes or whole cells that control biogenetic or biocatalytic processes,
3) Incorporation and release of bioactive compounds capable of diffusion, *e.g.* suppressing biological processes either on the surface or in neighbouring phases.

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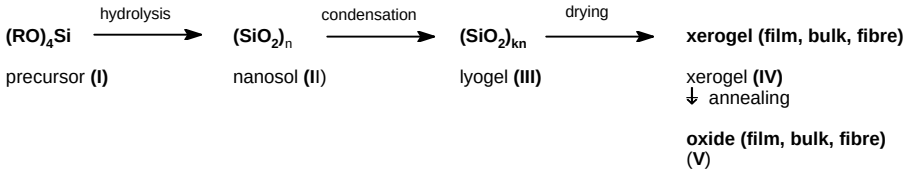
Bioactive layers need to have either specific surface structures or offer the effective immobilization of one or more bioactive components. Thin metallic oxide layers prepared by sol-gel processes meet these needs. Inorganic oxide layers offer important advantages when combined with biological systems:

– Good mechanical, thermal and photochemical stability
– Swelling independent on pH
– Preparation at room temperature
– High spectral transparency as far as the deep UV region
– Not a food source for microorganisms, since they are toxicologically and biologically inert
– Wide range of layer porosity and the degree of immobilization of embedded biocomponents.

The general procedure for preparing sol-gel materials is as follows (see also Fig.1):



Scheme 1 Silica sol-gel process

Inorganic nanosols (**II**) can be produced by either acid or alkali catalyzed hydrolysis of the corresponding silicon or metal alkoxides **(I)** in water or any organic solvent miscible with water (usually ethanol). They are transparent, stable nanoparticular dispersions with solid content between 4 and 20wt-%. After being neutralized, the sols condense (or gel) to form solvent-containing lyogels (**III**) when temperature or concentration is increased. Once dried the result is a porous xerogel (**IV**) in the form of a powder or film. Thus inorganic xerogel films are formed on the substrate by the liquid sol film solidifying as a result of solvent evaporation. When annealed at high temperatures, pure oxide films can be obtained.

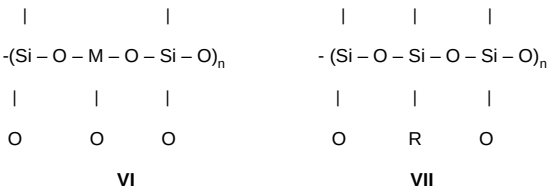
The conditions for hydrolysis and drying govern the density, porosity, critical thickness for cracking, and the mechanical properties of the layers [11–16]. One reason for this is the dependence of gel structures on the pH-value of their corresponding sols: acid hydrolysed sols gel to linearly cross-linked condensation products with a denser layer structure whereas alkaline sols form particle-like polymer films with larger pores [4, 17]. Studies show that for a wide deformation range (10–600 cm^{-1}) the rheology of nanosols is similar to Newtonian liquids [18], i.e. their viscosity is independent of shear rate. Therefore, thin sol-gel films can be produced without any problems using conventional deposition technologies. After coating any substrate, *e.g*. by dip coating [12, 19], spin coating [20], spray coating [21] or continuous casting [18], the results after drying are strongly adhering transparent films with layer thickness between 0.1–2 µm. However cracking defects may occur for thicker layers (> 2 µm), which does not occur in polymeric layers. This can be avoided by using multiple coating techniques or by chemically or physically modifying the oxide matrices with the addition of filling materials such as inorganic pigments or organic polymers [9].

Three special features help explain why the sol gel-technique is of such interest:

1) Diverse applications (bulk products, films, fibres, and extremely porous aerogels)

2) The sol-gel matrix can be chemically modified by co-hydrolysis and co-condensation using

i) various metal oxides when preparing mixed oxide layers (**VI**)



with M = Al, Ti, Zr, Sn, B, P, and others, or/and

ii) alkoxysilanes to produce organic modified silica **(VII)**, where R is *e.g*. alkyl, dye, polymer or a biomolecule (organically modified ceramics (ORMOCER®s [22, 23]). In this way a direct covalent linkage from any organic or biological residues to the oxide matrix can be realized.

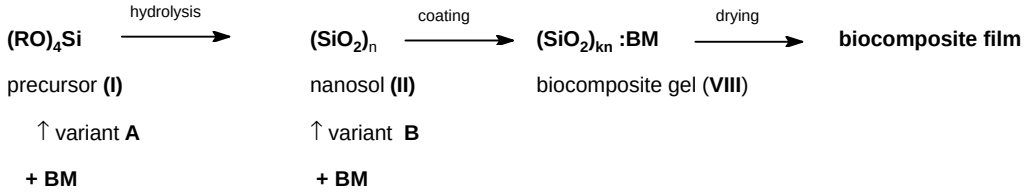
In case the precursors of mixed nanosols have highly different hydrolysis rates, rate coordination by selective complexation of the more reactive component is required.

The covalent linkage of a BM with the oxide matrix offers a possible method for preparing bioactive sol-gel layers. Compared with physical immobilization, chemical fixing requires expensive preparative work and special reactive functionalities either on the sol or the biological component. A similar method is the supplementary covalent binding of proteins (*e.g*. antibodies) to the surface of functionalized silica films [24].

3) Physical modification of the sol-gel matrix is the most important method of preparing bioactive sol-gel coatings. This simple method allows almost any organic substance (drugs, oils, dyes, polymers) or BM to be incorporated within the sol-gel matrix by adding the ingredients either before (variant A) or after (variant B) hydrolysis of the precursors (see Scheme 2):

In many cases the composite structure and immobilization behaviour of both variants are identical since encapsulation actually occurs at the condensation step.

Figure 1 illustrates again the simple way in which the embedding of BMs within the sol-gel oxide matrix can be used to prepare bioactive layers and bulk products. The immobilization of BMs within the inorganic



Scheme 2 Preparation of biocomposite films (BM = biomolecule)

Table 1 Selected examples of sol-gel matrices with embedded biocomponents

Biocomponent	Topics	Ref.
a) enzymes (see also tables in [35, 38, 47])		
glucose oxidase	glucose biosensors	[26–40]
	structure investigations	[41]
peroxidase	H_2O_2 sensor	[42–44]
oxalate oxidase	oxalate sensor	[32, 33]
trypsin	enzyme activity	[27, 45–47]
phosphatase	enzyme activity	[35, 46, 47]
tyrosinase	phenol biosensor	[48]
urease	urea sensor	[49, 50]
superoxid-dismutase	de/remetallization	[33, 51, 52]
nitrate reductase	determination of nitrate	[53]
lipases	catalytic hydrolysis of rapeseed oil	[54]
	biocatalysts	[55]
formate dehydrogenase	CO_2 reduction	[56]
b) other proteins		
cytochrome c , hemoglobin	redox catalyst	[33, 52]
myoglobin	CO oxidation	[51, 57]
	CO, NO, O_2 sensor	[32, 58]
bacterio-rhodopsin	optical imaging and sensing	[33, 59, 60]
bovine serum albumin	structure investigation	[61, 62]
monellin	structure investigations	[63, 64]
scleroproteins	gel structure investigations	[65]
	biocompatible coatings	[66]
antibodies	immunosensors	[67]
	ELISA of pesticides	[68]
	nitroaromatics sensor	[69]
	antigen reactions	[70, 71]
c) cellular systems		
yeast cells	biocatalyst	[72, 73]
	phenol and PCB biodegradation	[74]
	accumulation of metal ions	[75]
pancreas islets	artificial pancreas	[76]
plant cells	production of metabolits	[77]
	production of alkaloids	[78]
animal cells	preparation of ”biosils”	[79]
	bioartificial organs	[80]
parasites	immunoassays	[81, 82]
bacillus sphaericus	metal accumulation	[83]
E. coli bacteria	enzymatic activity	[84]

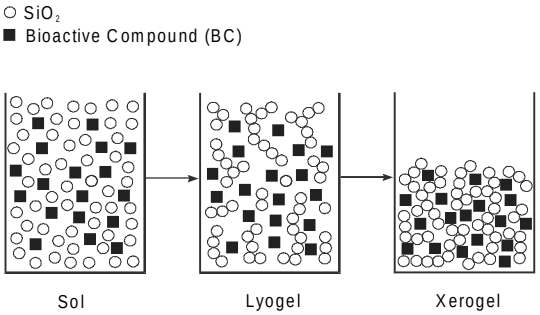


Fig. 1 Scheme of sol-gel embedding of bioactive compounds (after [129])

matrix is very efficient and can be controlled by the BM:oxide ratio and by addition of penetration agents [25, 26].

2. Properties of Bioactive Sol-Gel Material

The embedding of biological molecules in sol-gel matrices has received considerable attention because of the creation of novel biocomposite materials exhibiting the characteristic chemical and biochemical functionalities of enzyme, protein and other biocomponents.

Table 1 demonstrates the universality of this method of embedding biocomponents in sol-gels since almost all types of biocomponents can be embedded. By avoiding preparation conditions that would lead to denaturising (extreme pH-values, high levels of organic solvent in the nanosol), even cellular systems (such as bacteria, yeast cells, or Langerhans islands) can be embedded and still maintain their viability.

In the same way that organically-modified ceramics are termed *ormocers*, these bioactive ceramic composites can be described as *biocers*, though in both cases it should be noted that the inorganic matrix corresponds to a ceramic only in its chemical composition and not its solid state structure.

To a large degree the embedded BMs retain their conformation, chemical and physical properties, displaying activities approaching those of the free molecules together with their high stability and robustness, i.e. the sol-gel matrix stabilizes the embedded proteins against chemical and thermal denaturization. For example, encapsulating myoglobin in a sol-gel results in the active form remaining stable in conditions of high temperature and extreme pH values that would normally denature the protein [85].

Due to the porosity of the nanostructured sol-gel matrix, the embedded molecules are easily accessible to external reagents and chemical reactions can proceed within the inorganic layer with a high speed, *e.g.*, reaction rates with embedded enzymes are comparable with those in aqueous solutions, see section 3.2. Moreover, that redox reactions are entirely reversible was proved with encapsulated cytochrome c [86]. Normally, large BMs are permanently immobilized within the oxide matrix and only small molecules are able to diffuse and react. Varying the preparation conditions allows the pore-size of the sol-gel matrix to be controlled in such way that reactions with large protein molecules can also proceed [38].

Even gels doped with antibodies retain their ability to bind free antigens from aqueous solution [68].

3. Applications for Bioactive Sol-Gel Coatings

Depending on the degree of immobilization and the function of the biocomponent in the bioactive sol-gel layer, there are three fundamental fields of application as illustrated in Figure 2:

1) Layers where the immobilized biocomponent has changed the layer surface and structure in such a way that biological growth processes (biomineralization, biogenous processes) are initiated or promoted (template effect).

2) Layers where the immobilized biocomponent is able to catalyze biochemical reactions that can be used in biosensors and for biocatalysts.

3) Layers where controlled release of the embedded bioactive compound promotes or suppresses biological growth processes on or surrounding the coating surface.

These options are explained in greater detail below.

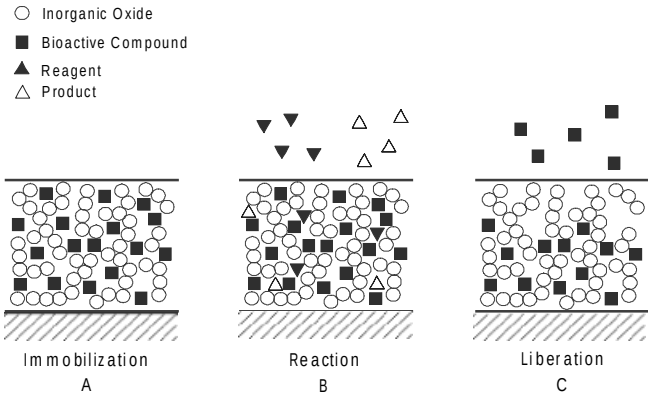


Fig. 2 Fundamental applications of bioactive sol-gel coatings

3. 1. Biocompatible Sol-Gel Coatings

Biocompatibility is defined as the ability of a biomaterial to perform with an appropriate host response in a specific application [87]. The goal is to produce materials which can be smoothly integrated into living systems instead of fighting them [88].

Biocompatible deposits are of specific interest in generating biogenous surfaces (*e.g.* for the cultivation of artificial organs and in tissue engineering) as well as

for coating medical products, in particular implants to support the new formation of bone (itself a composite material consisting mainly of two components, collagen I and hydroxyapatite). The aim is to fabricate matrices that both support and control mineral deposition to create an artificial composite ceramic with the physical characteristics of bone without the interface of living cells (biomineralization [2, 89–91]). Moreover, the materials chosen for implants should be non-toxic and non-antigenic while allowing the growth of immune and phagocytic cells, and of native tissue.

Sol-gel layers consisting of silica and various metal oxides are already biocompatible, i.e. they promote the deposition of hydroxyapatite (HAP) from natural or simulated body fluids [92–99]. At body temperature (37 °C) sol-gel prepared silica films induce HPA-deposition from metastable calcium phosphate solutions ("simulated body fluids"). Titania shows a similar behaviour for having a negatively charged surface, but not alumina with its positively charged surface [94].

More detailed investigations [95–97] reveal that reactive OH groups on the silica surface reduce the HPA nucleation energy and initiate the deposition of Ca/P phases. Therefore, such layers are of special interest for coating bone implants because they support the regeneration of bone directly on the implant surface. Now it seems possible to integrate further bone-relevant components such as calcium, phosphate and proteins into the sol-gel layers to additionally improve the biocompatibility as well as controlling and modulating the nucleation and growth of biomineralization processes.

The immobilization of different bone-relevant proteins such as collagen, gelatine and commercial collagen hydrolysates within the silica sol-gel coating can be realized without any problems by avoiding high alcohol concentrations in the sol, using instead *e.g.* sols prepared in aprotic solvents such as dioxane, or aqueous sols produced by the careful distillation of alcohol from the sols and the simultaneous addition of an adequate amount of water [66, 100].

The biocompatibility of the coatings was tested by contacting them with specific cell cultures (*e.g.* fibroblasts or osteoblasts) and assessing how the deposit promoted cell adhesion and proliferation.

Figure 3 compares the mechanical and biofunctional properties of different sol-gel coatings with those of a pure uncoated titanium implant surface. Even a pure silica layer has improved cell adhesion (the most important criterion of the biocompatibility) when compared with the titanium layer. The additional incorporation of further bone-relevant components such as phosphate, gelatine, and calcium salts gradually improves cell adhesion. In contrast with the polymeric and metallic implant materials used today, the mechanical properties of the biocompatible sol-gel coatings (hardness H and

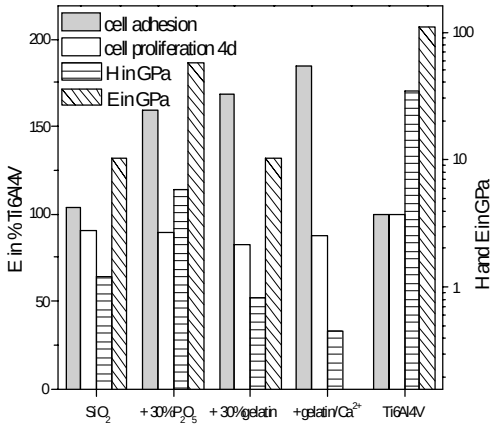


Fig. 3 Properties of different sol-gel layers for implant coating

elastic modulus E) are in the same range as natural bone, whereas layers with embedded gelatine in particular show a high abrasion resistance. *In-vivo* investigations of selected composite coatings are in progress.

The investigations are a first step in achieving implant coatings with a higher biocompatibility by integrating natural proteins. The incorporation of additional functional BMs (*e.g.* antibiotics, bone morphogenetic proteins [101], and other growth factors for bone regeneration [102]) offers new opportunities for improving the biocompatibility of implants and of enhancing their long-term stability.

3.2. Biocatalytic Sol-Gel Coatings

The utilisation of heterogeneous biocatalysts for analytical purposes [103] and biotechnical applications [104] requires the development of immobilization methods capable of providing cheap, stable, and efficient materials. The sol-gel procedure permits the immobilization of biocomponents within a mechanically stable porous matrix, where diffusion processes and reactions between the biocomponents and gases or small dissolved molecules and ions can be very efficient. Examples are the de- and re-metallization of sol-gel embedded Cu-Zn superoxide dismutase, reversible redox processes with cytochrome c and myoglobin [51], and the complexing of immobilized bacteriorhodopsin with Ca^{2+} salts [59].

Combining the high reactivity of immobilized biomolecules within the sol-gel matrix with the possibility of deposition onto any substrate opens new perspectives for biosensors and technical biocatalysis [32, 35, 105–107]. For these applications the immobilization of enzymes is of special interest since they catalyse very selectively the conversion of a substrate and do not burn themselves out during the reaction, meaning that they can be reused without regeneration.

The systems listed in Table 1 concentrate on the immobilization of *glucose oxidase* (GOD) [108], widely

used as an analytical reagent in the medical diagnostic and food industries.

Using steady-state and time-resolved fluorescence measurements, investigations into the kinetics and thermodynamics of free flavins and of the flavin-based redox active site of GOD entrapped within a sol-gel silica matrix revealed that GOD molecules do not leach from the glass and that their rotational mobility is only half that in aqueous solution, whereas the folding kinetics of redox active site flavin adenine dinucleotides (FAD's) are 3–10 times slower within the glass than in aqueous solution [41].

Glucose oxidase catalyzes the oxidation of glucose by atmospheric oxygen:

$$\beta\text{-D-Glucose} + O_2 + H_2O \xrightarrow{GOD} \text{gluconic acid} + H_2O_2$$

Scheme 3 Enzymatic oxidation of glucose

From scheme 3, the glucose can be determined quantitatively by:
i) determining of the oxygen concentration [28],
ii) calorimetric measurement of the reaction heat [109],
iii) amperometric or voltametric measurement of H_2O_2 concentration using sol-gel coated electrodes [107]. To further improve response and reproducibility, redox mediators such as ferrocens or quinones are often embedded within the sol-gel matrix [26, 31, 34, 36, 39], optical sensing, *e.g*. after the formation of optically absorbing compounds from the peroxidase (POD) catalyzed oxidation of dye precursors by hydrogen peroxide in co-immobilized systems based on coupled enzymatic reactions (Scheme 3 and 4) [27, 30]:

$$H_2O_2 + \text{dye precursor} \xrightarrow{POD} \text{dye}$$

Scheme 4 Enzymatic dye formation

Thus prototypes of glucose biosensors combine the immobilization of GOD in different layer arrangements with electrochemical and optical transducers [105, 107]. The performance of glucose biosensors depends decisively on the activity of the immobilized GOD and on its storage stability. Usually the enzyme activity can be characterized by the Michaelis constant K_m, which can be determined from the relationship between reaction rate and substrate concentration (Michaelis–Menten equation [110]). K_m is a complex rate constant and is defined as half of the substrate concentration for reaching the maximum reaction rate. K_m values between 10^{-2} – 10^{-5} mol/l are found in solutions, and the smaller the value of K_m, the greater the affinity between GOD and substrate glucose.

Table 2 shows some Michaelis constants for SiO_2–GOD composite layers with reference to the method of sol preparation [26]. The following conclusions can be drawn:
· The K_m values are comparable with those for GOD dissolved in phosphate buffer solution (K_m 0.01–0.1 mmol l^{-1}).
· Even after leaching in water, considerable GOD activities are registered.
· Although 60% of activity is lost within the first 24 hours as a result of the consolidation of the silica matrix, the remaining activity stays constant for several months.

The GOD activity depends strongly on the type of sol-gel matrix [26, 27, 30, 31]: Activity decreases when the immobilization is in an incompletely hydrolyzed sol (*e.g*. when the molar ratio H_2O:$(RO)_4Si$ is smaller than 2), when sols with high alcohol concentrations are used, and in organically modified silica matrices.

Enzyme activity increases with the use of so-called penetration agents [25, 26, 111]. Penetration agents are highly-soluble compounds, *e.g*. saccharides, co-immobilized within the sol-gel matrix that enhance porosity by leaching during the enzyme reaction. In this way they improve the diffusion of reactants within the metal oxide matrix both towards and away from the embedded GOD. BET surface and pore volume increase linearly with the concentration of the added penetration agent ("non-surfactant templating" [112, 113]).

Based on these results, prototypes for various electrochemical glucose sensors have been developed [26]. The effects of the sol gel-matrix on enzyme activity vary with the type of enzyme being immobilized. Many investigations have been carried out into sol-gel immobilized *lipases*, the enzymes used most frequently in organic syntheses [54, 55]. Lipases are very efficient biocatalysts for hydrolysing esters (technically important for splitting fats), but in organic solutions the biocatalysis of esterification and transesterification is also possible. Since lipases are difficult to remove from the reaction mixture, the enzymes must be immobilized on

Table 2 Enzyme activities of GOD/SiO_2 composite layers [26]

SiO_2 sol	catalyst	solvent	wt-% GOG	layer thickness/nm	K_m mmol/l [a])	K_m after leaching [b])
1	0.05M NaOH	70% EtOH	48	460	0.04	0.09
2	0.005M HCl	70% EtOH	48	500	0.03	0.13
3	0.005M HCl	70Dioxan	48	520	0.04	0.24
4	4 % TEA [c])	water	38	490	0.11	no activity

[a]) K_m in phoshate buffer solution 0.01...0.1 mmol/l [b]) 20 min leaching in stirred water [c]) TEA = triethanolamine

the reactor surface or on inert carriers.

Lipases can also immobilized very efficiently in sol-gel matrices (degree of immobilization 90–95%, enzyme activity after 30 reaction cycles 80–85% [55]). The entrapping takes place close to the surfaces of the gel particles, where substrate molecules are readily accessible.

The relative activity of the immobilized enzyme in the model reaction (esterification of lauric acid by *n*-octanol in isooctane) in pure silica (by hydrolysis of TEOS) is just 3–29% of that of the non-immobilized enzyme suspension, but surprisingly the activity increases strongly with increasing hydrophobicity of the sol-gel matrix (*e.g.* by a factor of 13 when compared with the non-immobilized enzyme suspension in pure gels from hydrolyzed CH_3–$Si(OCH_3)_3$). Moreover, the relative activity rises sharply in gels with increasing lipophilicity in the order R = Me < Et < Pr < Bu, obtained by hydrolysis of R–$Si(OR)_3$ mixed with $Si(OCH_3)_4$. It is probable that lipophilic domains in the enzyme are stabilized and activated by the hydrophobic alkyl groups R in the sol-gel matrix ("alkyl effect") [55].

For biocatalytic applications, the coating of lipase-containing sols on sintered glass beds such as SIRAN® (Schott Engineering GmbH) or controlled-pore glass has proved to be the optimum method. These highly active and mechanically stable biocatalysts are very effective in fluid-bed reactors and can be re-used many times without marked loss of enzyme activity [114].

Table 1 shows uses of the sol-gel technique for efficiently immobilizing living cells, and their applications in biotechnological systems. For example, viable yeast cells are immobilized into silica sol-gel layers coated on glass. The immobilized biocatalyst survives experimental processing and can be successfully applied *e.g.* for the degradation of sucrose [73].

It seems that these first successful results may be the starting point for a new generation of biomaterials ("living composites").

3.3. Sol-Gel Coatings with Controlled-Release Bioactive Compounds

The possibility of embedding bioactive compounds into inorganic oxide matrices using the sol-gel technique offers new and interesting perspectives for coatings with controlled release effects. Such systems could be used for different therapeutic and antibacterial depot systems, where the metal oxide is only an inert carrier for the diffusible bioactive compound, see Table 3.

To date, controlled release systems [122] using sol-gel matrices with incorporated BCs are still largely unknown. Only a small number of studies have been made into the diffusion of drugs such as nifedipin [120], antimicrobial substances [25, 115], and steroids [123] from porous sol-gel glasses, and into the release of perfumed essences and oils from hybrid SiO_2 lyogels [124], xerogels, and films [118].

The release of embedded BCs from the sol-gel matrix into the adjacent gas or liquid decreases with increasing mass ratio of silica:BC, layer thickness, molecular mass or the size of the BC. The releasing behaviour also depends on the degree of interaction between the BC and the layer matrix, and on the porosity of the inorganic matrix resulting from the drying regime and additives used. The pore volume determines how much of the embedded BC is available for release, while pore diameter affects the strength of retention since the diffusion rate for small pores depends on cavity size and increases with diameter. In many cases, the liberation curves of bioactive compounds from sol-gel coatings [25, 115, 118] suggest two different processes are oc-

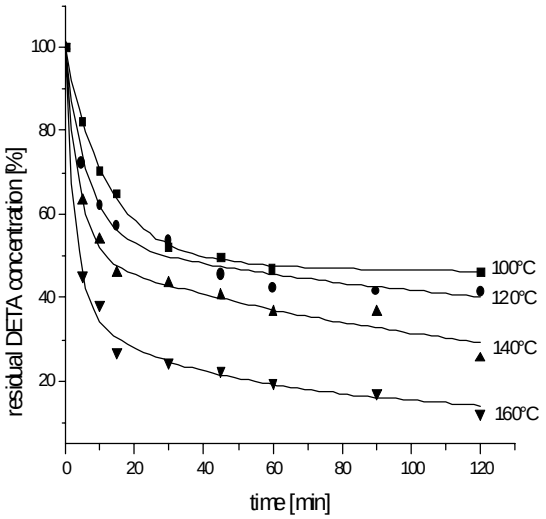


Fig. 4 Release of the insect repellent diethyltoluamide (DETA) from silica layers at different temperatures [118]

Table 3 Examples of sol-gel coatings for the controlled release of embedded bioactive compounds

Embedded bioactive compounds	Application	Ref.
benzoic acid, sorbic acid	preservation of foods	[25, 115]
boron compounds	consolidation and protection of wood	[115, 116]
biocides	antimicrobial coatings	[115–117]
diethyltoluamide, citronella, mint oil	insect-repellent coatings	[118, 119]
dexpanthenol, vitamin E	dermale systems	[111, 119]
drugs	retarding drugs, transdermal systems	[111, 120]
heparin, hirudin	anticoagulating coatings	[121]

curring: initial fast release of the BCs from open pores connected directly with the layer surface followed by much slower discharge from internal pores. Figure 4 shows the thermal release of the bioactive liquid insect repellent diethyltoluamide (DETA) at different temperatures from a silica film. At 100 °C only about 50% of the embedded liquid can be set free, whereas the complete release of embedded DETA from internal pores requires temperatures over 160 °C [118].

Release can be controlled by modifying the sol-gel matrix with additives. While adding water-insoluble polymers reduces the releasing rate, the addition of soluble or swelling penetration enhancers such as sugars or cellulose derivates accelerates the rate as a result of enlargement of the pore structure [25, 115].

Knowledge of these relationships allows the release of bioactive ingredients from sol-gel coatings to be controlled in such a way that numerous promising applications are possible (see Table 3). The first commercial products, *e.g.* for wood preservation, are already available [125]. It should be noted that the controlled release of volatile corrosion inhibitors (VCIs) from sol-gel layers is already used commercially to a considerable extent [126].

In practical applications, bioactive sol-gels coatings offer two further advantages: The coatings are well suited for the functionalisation of flexible supports such as paper, textiles, and polymers as they exhibit good adhesion and additionally improved mechanical properties.

It is possible to combine and co-immobilize several bioactive components, and combinations with other functional compounds are possible. Such multifunctional coatings are a prerequisite for the production of "smart materials".

The multifunctional properties of bioactive sol-gel coatings can be demonstrated with respect to the protection of wood. Impregnating pin wood with pure silica sols improves mechanical properties (*e.g.* increased Brinell hardness, decreased swelling, better dimensional stability) while reducing flammability. Further, resistance to fungi and insects is increased (see Table 4).

The antimicrobial effect can be enhanced by the incorporation of boric acid (BA). BA is one of the most effective and least toxic agents for protecting wood against brown and white rot, the larvae of the house longhorn beetle (*Hylotrupes bajulus*) and the common furniture beetle (*Anobium punctatum*). Use of this biocide is however mainly limited to wood that will not be exposed to rain or high humidity, due to its solubility in water and inability to be fixed. By incorporating BA within modified silica coatings, the release of BA can be controlled *e.g.* by simultaneously embedded complexing agents such as polymeric polyols or precursors that only liberate BA in the presence of moisture [127]. Testing the biocidal activity of 30% w/w BA within the silica (see Table 4) illustrates the total inhibition of growth of *Coniophora puteana*. Even after 20 weeks of incubation no decrease of mass was observed. The termite test resulted in 100% mortality with no decrease in mass [115]. The results of both the leaching and microbiological investigations combined with the low ecotoxicity of the compounds under consideration suggest that these composite coatings have great potential as ecologically beneficial wood preservatives.

Other prospective fields of application for bioactive sol-gel coatings with controlled release behaviour could be antimicrobial coatings for food-packaging materials (foils, papers) and for medical equipment such as catheters and instruments. The release rates for food preserving agents (benzoic, sorbic and boric acids) incorporated into modified silica films correlate with their biocidal activity, i.e. the growth of microorganisms such as *Escherichia coli, Lactobacillus plantarum,* and *Penicillium sp*. is strongly suppressed by contact with such composite films [115].

To overcome the problem of catheter-related infections, the application of antimicrobial substances to the surface of the device has been suggested [128]. Antimicrobial sol-gel coatings [117] offer new possibilities because they can be coated onto any cheap polymeric material. Moreover by embedding a tuned mixture of biocides and antibiotics, long-term effects with a broad antimicrobial spectrum can be obtained.

4. Conclusions

The results demonstrate that the sol-gel technique is a versatile new method for embedding and immobilizing bioactive compounds within an inorganic oxide matrix and for the preparation of new bioactive coatings. The sol-gel immobilization offers an interesting alternative to the present widely used techniques with organic polymers or supermolecular carriers. The sol-gel matrix is

Table 4 Biocidal activity of boric acid (BA) containing silica sols [115]

Sol	Coniophora puteana		Reticulitermes sanonensis			
	Decrease of mass (%)		Decrease of mass (%)		Mortality (%)	
	treated wood	untreated wood	treated wood	untreated wood	treated wood	untreated wood
pure $Si0_2$	0.5	42.0	13.3	26.2	49.0	0
+ 30% BA	0	26.6	0	26.2	100	0
+50% BA	0	19.4	0	26.2	100	0

transparent, inert, non-toxic, stable to heat and light, and forms stable coatings on such varied substrates as polymer foil, paper, tissue or wood. Oily and high viscosity substances of pharmaceutical interest can also be incorporated without problems. All embedded biocomponents show high biological activity. It can be expected that the wide variety of sol-gel matrices and bioactive components will create new and interesting bioactive systems and prospective applications.

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