Molecular Crystals and Liquid Crystals Science and Technology. Section A. Molecular Crystals and Liquid Crystals

Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/gmcl19

Bio-Encapsulation Within Sol-Gel Glasses

Laurie Bergogne [a], Souad Fennouh [a], Stephanie Guyon [a], Jacques Livage [a] & Cecile Roux [a]

[a] Chimie de la Matière Condensée, Université P.M. Curie, 75052, Paris, France

Version of record first published: 24 Sep 2006

To cite this article: Laurie Bergogne, Souad Fennouh, Stephanie Guyon, Jacques Livage & Cecile Roux (2000): Bio-Encapsulation Within Sol-Gel Glasses, Molecular Crystals and Liquid Crystals Science and Technology. Section A. Molecular Crystals and Liquid Crystals, 354:1, 79-89

To link to this article: http://dx.doi.org/10.1080/10587250008023605

Mol. Cryst. and Liq. Cryst., 2000, Vol. 354, pp. 79-89
Reprints available directly from the publisher
Photocopying permitted by license only

Bio-Encapsulation Within Sol-Gel Glasses

LAURIE BERGOGNE, SOUAD FENNOUH, STEPHANIE GUYON, JACQUES LIVAGE and CECILE ROUX

Chimie de la Matière Condensée, Université P.M. Curie, 75052 Paris – France

The sol-gel process opens new possibilities in the field of biotechnology. Enzymes such as glucose oxydase and lipase can be trapped within sol-gel glasses without loosing their bioactivity. Small analytes diffuse through the pores of the silica matrix allowing in-situ biocatalytic reactions to be performed. *Escherichia coli* bacteria have also been encapsulated. Their cellular organization is preserved and they still exhibit noticeable enzymatic activity, even on dried xerogels. Antibody-antigen recognition has been shown to be feasible within sol-gel matrices and whole cell protozoa have been used as antigens for blood tests with human sera.

Keywords: sol-gel; enzymes; bacteria; bio-encapsulation; immunoassays

INTRODUCTION

The immobilization of enzymes is a key point for biotechnology. Immobilization can be performed via adsorption, covalent binding or entrapment [1]. Organic polymers are currently used for immobilization but silica glasses could offer some advantages such as improved mechanical strength and chemical stability. Moreover they don't swell in most solvents preventing the leaching of entrapped biomolecules. However glasses are made at high temperature and up to now enzyme immobilization can only be performed via adsorption or covalent binding onto the surface of porous glasses [2].

Soft chemistry routes known as "chimie douce" have been developed during the past two decades. They are performed in solution under ambient conditions and open new possibilities for materials chemistry. The so-called "sol-gel process" is based on the inorganic polymerization of metal alkoxides. Organic and inorganic molecular precursors can be mixed together leading to

the formation of to hybrid nanocomposites. Such organic-inorganic hybrids have already been developed for almost twenty years by both polymer and sol-gel chemists [3]. The sol-gel encapsulation of biological species within silica matrices was reported much later [4]. It has been shown that enzymes, trapped within a porous oxide matrix, retain their biological activity and could be used as biocatalysts or biosensors [5][6]. Many papers have been published since then extending the field to a wide range of biological species such as antibodies and even whole cells [7]. This paper discusses the main advances of the sol-gel process in the field of biotechnology.

SOL-GEL SYNTHESIS OF SILICA

The sol-gel synthesis of silica is based on the hydrolysis and condensation of silicon alkoxides $Si(OR)_4$ where R is usually an alkyl group (R = CH_3, C_2H_5,...) [8]. Hydrolysis gives reactive Si-OH groups whereas condensation leads to the formation of oxo bridges Si-O-Si.

$$Si\text{-}OR + H_2O \Rightarrow Si\text{-}OH + ROH \qquad \text{(hydrolysis)}$$

$$Si\text{-}OH + RO\text{-}Si \Rightarrow Si\text{-}O\text{-}Si + ROH \qquad \text{(condensation)}$$

The overall reaction can be written :

$$Si(OR)_4 + 2H_2O \Rightarrow SiO_2 + 4\ ROH$$

Alkoxides are not miscible with water and a common solvent, usually the parent alcohol ROH, has to be added. Silicon alkoxides are not very reactive toward hydrolysis and condensation. Gelation could take several weeks and sol-gel chemistry is currently performed in acid or basic conditions. Hydrolysis rate increases with acid catalysis whereas condensation goes faster with basic catalysis. Catalysis does not only increase reaction rates, it also controls the shape of silica particles and the porosity of the gel. Chain polymers are formed at low pH giving a microporous gel (pore diameter < 2nm). Dense particles are formed in basic conditions and a mesoporous gel is obtained (pore diameter > 5nm) [8].

The chemical conditions associated with sol-gel chemistry (metal-organic precursor, organic solvent, room temperature) are close to those of organic chemistry allowing the synthesis of hybrid organic-inorganic compounds. However they are not gentle enough for biological molecules such as proteins. The sol-gel process has then to be slightly modified in order to fit the requirements of biochemistry [9].

i) Proteins are denatured by alcohols, methanol being the less harmful. Therefore the tetramethyl orthosilicate (TMOS = $Si(OCH_3)_4$) is taken as a precursor and water is added directly without any alcohol as a co-solvent. Both non miscible liquids are vigorously mixed (often via sonication). Some acid (HCl) is usually added in order to increase hydrolysis rates leading to the fast formation of fully hydrolyzed precursors $Si(OH)_4$.

ii) Proteins are usually not stable outside a narrow pH range around pH 7. Therefore they are kept in a buffered solution to which hydrolyzed precursors are added. The pH buffer being close to 7, basic conditions are obtained, condensation is quite fast and a silica network is formed around the biomolecule. The size of the pores is in the range 1-10 nm in diameter allowing the diffusion of small molecules into and out of the porous glass.

ENZYME ACTIVITY WITHIN SOL-GEL GLASSES

Enzymes are biological catalysts which are responsible for the chemical reactions of living organisms. Their high specificity and huge catalytic power is due to the fact that the geometry of the active site can fit exactly that of the substrate. The immobilization of enzymes on porous glasses has already been developed for more than twenty years. Enzymes are covalently bonded via organosilane coupling agents such as γ-aminopropyltriethoxysilane, H_2N-$(CH_2)_3$-Si-$(OEt)_3$ [2]. However such covalent attachments requires chemical modifications that may affect the catalytic activity of enzymes. Therefore the physical encapsulation within sol-gel glasses could offer new possibilities in biotechnology.

Many enzymes have already been embedded within sol-gel matrices (Table I). Glucose oxidase (GOD) is by far the most studied enzyme and has often been used as a model for sol-gel encapsulation It catalyzes the oxidation of glucose by molecular oxygen and finds many applications in the field of medicine and food industry.

The oxydation of glucose can be written as follows:

$$C_6H_{12}O_6 + O_2 \Rightarrow C_6H_{10}O_6 + H_2O_2$$

The formation of hydrogen peroxide can be followed by optical measurements using another enzymatic reaction in which the oxidation of an organic dye is catalyzed by a horseradish peroxidase [10][11].

Table I. Sol-gel encapsulation of enzymes [6][7]

enzyme	sol-gel matrix
acid phosphatase	SiO_2
alkaline phosphatase	SiO_2
aspartase	SiO_2
L-amino acid oxidase	TiO_2
glucose oxidase	SiO_2, V_2O_5,
hydrogen peroxidase	SiO_2, V_2O_5
Cu-Zn superoxide dismutase	SiO_2
catalase	SiO_2
chitinase	SiO_2
urease	TiO_2/ZrO_2
trypsin	SiO_2
lipase	SiO_2-$RSi(OMe)_3$

The presence of glucose in the solution can also be followed via the electrochemical detection of redox reactions at the active site of GOD. However, GOD is a bulky enzyme and electrons cannot tunnel directly from the active site to the electrode. Molecular mediators such as ferrocene have to be added to allow electron transfer. The reduced mediator is regenerated at the anode and the current is proportional to the amount of converted glucose [12]. Electronically conductive matrices have been used in order to improve the response of amperometric biosensors. Carbon-ceramic composite electrodes (CCEs) in which enzymes are adsorbed onto carbon particles (electron conductor) dispersed into a rigid matrix (sol-gel silica) have been described recently [13][14]. The hydrophilic surface of silica can be organically modified in order to become hydrophobic. Water penetration is then limited to a very thin layer and the surface can be easily renewed by mechanical polishing.

In our group oxygen consumption was measured directly with an oxygen sensitive electrode. In these experiments the sol-gel solution containing GOD is deposited onto the Pt cathode of a Clark electrode between two polymeric films and placed into a cell containing the PBS buffer. The oxygen concentration is measured by amperometric titration at imposed potential and the enzyme activity is determined via the decrease in oxygen after the injection of D-glucose into the cell [15].

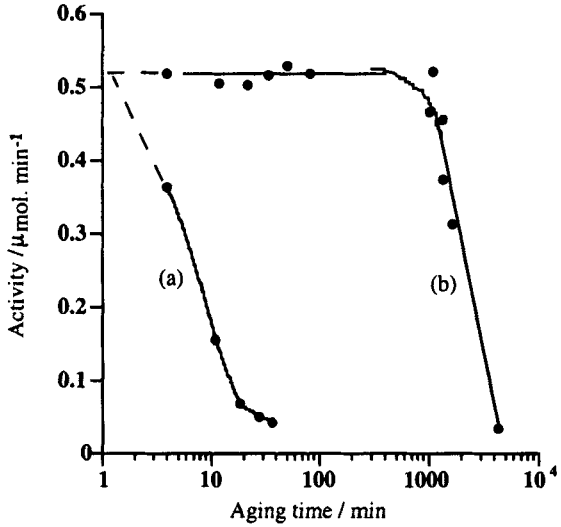

Figure 1. GOD activity as a function of time measured onto a Clark electrode (a) free enzyme, (b) encapsulated enzyme

A series of experiments was performed as a function of time. Between each titration, the electrode is washed carefully and dipped into a new buffered solution. Glucose is then added and the activity of GOD is measured. Figure 1 shows that the activity of entrapped enzymes is about the same as when enzymes are just deposited between polymeric films without sol-gel encapsulation. This suggests that the sol gel process does not lead to denaturation during the formation of the silica network. Even after drying at room temperature, silica xerogels still contain enough water to provide a mainly aqueous environment to avoid the denaturation of enzymes. The catalytic activity of the enzyme decreases more slowly with time when it GOD is encapsulated rather than just immobilized. The half-time $t_{1/2}$ (corresponding to a 50% decrease of the initial catalytic activity) is close to 50 hours for encapsulated GOD instead of 10 minutes when the enzyme is just immobilized. This suggests that the silica matrix protects the encapsulated enzyme against denaturation or leaching.

The sol-gel matrices does not only protect enzymes against denaturation. It can also provide a microenvironment that favors the enzymatic

reaction as shown by the encapsulation of lipases [16]. Lipases are widely used enzymes in organic syntheses [17]. They act on ester bonds and are able to hydrolyze fats and oils into fatty acids and glycerol. These are interphase-active enzymes with lipophilic domains and the catalytic reaction occurs at the water-lipid interface. Entrapped lipases can be almost 100 time more active when a chemically modified silica matrix is used. The co-hydrolysis of $Si(OMe)_4$ and $RSi(OMe)_3$ precursors provides alkyl groups that offer a lipophilic environment that could interact with the active site of lipases and increase their catalytic activity. Such entrapped lipases offer new possibilities for organic chemistry, food industry and oil processing.

ENCAPSULATION OF BACTERIA

Enzymes are currently prepared by extraction from animal or plant tissue or by cultivation of microorganisms such as bacteria. It could therefore be interesting to be able to encapsulate directly bacteria in order to avoid enzyme separation and purification procedures [18].

Whole cell bacteria *Escherichia coli* have been trapped within sol-gel glasses. They were first induced in their culture medium with IPTG (IsoPropylThioGalactoside) in order to express β-galactosidase. *E. coli* cells are extracted by centrifugation and suspended in a phosphate buffer solution (10^7 cells/ml). Sol-gel entrapment is performed via the usual two-step procedure. The pH of the prehydrolyzed acid solution of TMOS is first increased by adding a phosphate buffer in order to prevent the denaturation of bacteria cells. *E. coli* are added when the pH of the precursor solution is close to pH≈7. A gel is formed within few minutes and left for aging 3h at room temperature.

Ultrastructural observations by transmission electron microscopy of thin slices of wet gels show that the integrity of *Escherichia coli* cells is preserved after sol-gel encapsulation (Fig.2). The capsule, cell wall and plasma membrane are not destroyed. The porous texture of the silica matrix around entrapped cells can also be seen on these micrographs.

The β-galactosidase activity of entrapped *E. coli* was measured using p-NPG (p-nitrophenyl-β-D-galactopyranoside) as a substrate. It is cleaved by β-galactosidase into galactose and nitrophenol as follows:

$$\text{p-NPG} + H_2O \Rightarrow \text{p-nitrophenol} + \beta\text{-D-galactose}$$

The formation of the yellow p-nitrophenol is followed by optical absorption of the solution at $\lambda = 400$ nm.

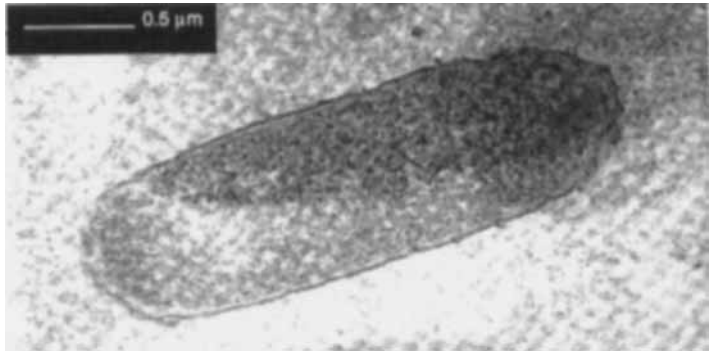

Figure 2.
Transmission electron microscopy of *E. coli* entrapped within a wet silica gel

The β-galactosidase activity of entrapped *E. coli* was measured on wet gels aged for 3 days and compared with the same concentration of *E. coli* cells suspended in the aqueous buffered solution. Figure 3 shows that entrapped bacteria exhibit a typical Michaelis behavior. The enzymatic activity increases rapidly with the substrate concentration [p-NPG] and reaches a maximum value V_{max}=1.1 µmol/mn. The corresponding Michaelis constant is K_M= 0.25 mM. These values are close to those observed with free suspensions of *E. coli* (Vmax≈0.9 µmol/mn, K_M≈0.45 mM). The β-galactosidase affinity for its substrateseems even better in gels. This might be due to the fact that bacteria are randomly dispersed in the gels whereas they form aggregates in the aqueous suspensions.

The β-galactosidase activity of entrapped E. coli can be preserved for many days as long as the gel remains wet despite the presence of a large amount of methanol. However it decreases significantly when the gel is dried in air at 37°C. The amount of water trapped in the gel appears to be a critical parameter, more than 70% in weight of water seem to be required to get a high activity.

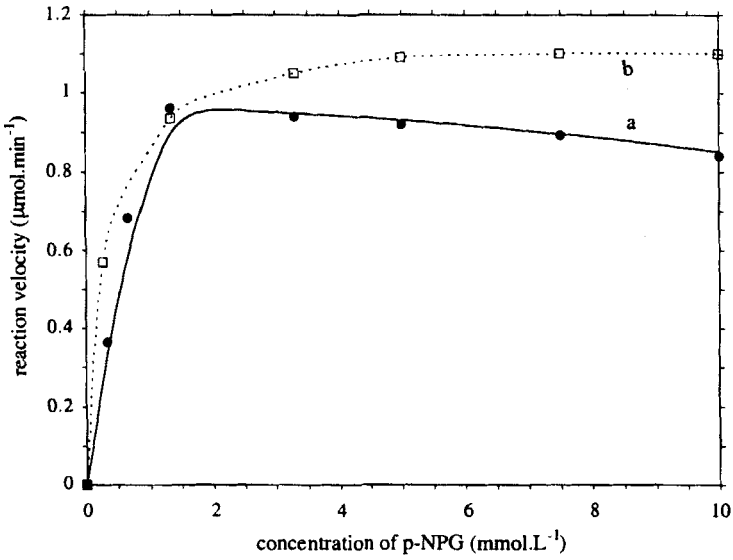

Figure 3. β-galactosidase activity of *E. coli*
(a) free cells, (b) cells entrapped within a wet gel

IMMUNOASSAYS IN SOL-GEL GLASSES

Antigen-antibody reactions have been performed within sol-gel matrices extending the field of sol-gel chemistry toward immunosensors [19]. The reactivity of an antibody is directed against a particular site on an antigen called antigenic determinant and the recognition of this determinant is highly specific.

However antibodies are large biomolecules that can hardly diffuse through the pores of a sol-gel matrix. Therefore experiments are performed with antibodies either bonded at the surface or trapped within the sol-gel matrix. Specific haptens are then used. They are much smaller and can diffuse easily through the pores of the sol-gel matrix. These reactions have been developed for the biodetection of chemicals such as atrazine, the most widely used herbicide. Anti-atrazine antibodies are trapped within the sol-gel matrix and nanograms of atrazine are applied on SiO_2 sol-gel columns doped with this

antibody. Titration of eluted solutions shows that high amounts of atrazine remain bound to anti-atrazine antibodies inside the silica gel [20][21].

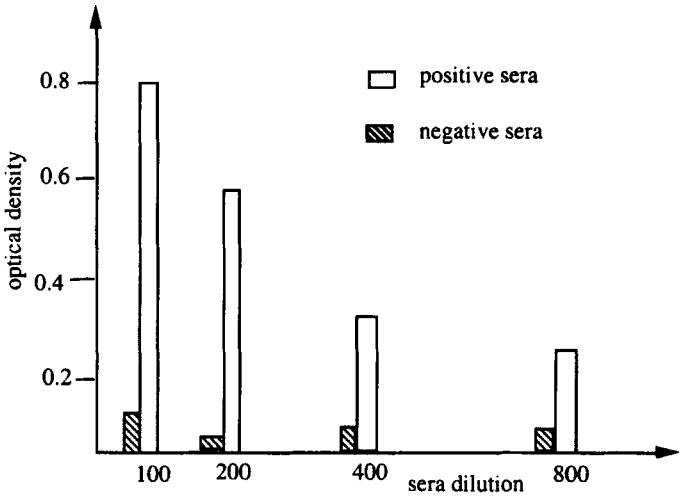

Figure 4. Optical density measurements of ELISA assays performed with encapsulated Leishmania cells and dog sera

For medical applications, whole cell parasitic protozoa (*Leishmania donovani infantum*) have been trapped within sol-gel matrices and used as antigens for blood tests with human sera [22]. As for bacteria, transmission electron microscopy shows that the cellular organization of the parasites is well preserved and that the plasma membrane is unaltered. This is very important as antigenic determinants are situated at the outside surface of the membrane. Antigen-antibody interactions were followed by the so-called Enzyme Linked ImmunoSorbent Assays (ELISA) which is now widely used in parasitology [23]. The presence of antibodies in the blood is detected via a colored reaction and optical density measurements show a clear difference between negative and positive sera (Fig.4). The optical density of positive tests decreases regularly with dilution showing that *Leishmania* cells have not been leached out by washing. Moreover immunoglobulins can diffuse through the mesopores of the sol-gel matrix and link specifically to the protein epitopes. Only non specifically bound immunoglobulins are washed out giving a very low residual coloration (optical density ≈ 0.03).

Several papers published during the last two years report on the entrapment of living cells within silica matrices. Yeast cells "*Saccharomyces cerevisiae* " have been immobilized into SiO_2 gels. They retain their bioactivity (conversion of sugar into C_2H_5OH and CO_2) for more than a year. Moreover silica-sol entrapment does not exclude viability and cell reproduction and the budding of yeast cells was still observed after immobilization [24].

Mammalian tissues such as the pancreatic islets of Langerhans have also been entrapped within sol-gel glasses [25] [26]. These cells are known to produce insulin in response of glucose. After encapsulation they have been transplanted into a diabetic mouse where they retain their activity. The fine porosity of the gel protects transplanted islets against antibody aggression but permits nutrients to reach the cell and by products to escape. Urinary excretion of glucose fell to almost zero within a few days of transplantation and remained close to zero for almost 3 months. In addition, both blood sugar and blood insulin concentrations were normal. After one month of transplantation, the surgically removed transplant showed no evidence of fibrosis.

CONCLUSION

The biological applications of sol-gel chemistry appear to be very promising. Enzyme immobilization within sol-gel glasses offer several advantages compared to organic polymeric matrices which are nowadays widely used in biotechnology. Biomolecules are trapped inside hard porous glasses that do not swell in water and protect biospecies against external aggression (pH, temperature, solvents...). The sol-gel encapsulation of enzymes has been extensively studied, patents have been taken and sol-gel lipases are now commercialized by Fluka.

Sol-gel encapsulation does not destroy the cellular organization of micro-organisms. This might be one of the major advance of sol-gel chemistry and some promising examples show that living cells can be immobilized within sol-gel matrices. Plant cells have been recently immobilized on glass substrates by sol-gel deposition. They could be used for the production of chemicals such as Taxol [27]. Biogel transplants, if viable for extended lengths of time, could also emerge as a viable treatment for diseases such as diabetes.

References

[1] R.G. Willaert, G.V. Baron, *Reviews in Chemical Engineering,* **12,** 1 (1996).

[2] H. Weetall, *Trends in Biotechnology,* **3,** 276 (1985).

[3] R.M. Laine, C. Sanchez, C.J. Brinker, E. Giannelis, in *Organic/Inorganic Hybrid Materials,* Mat. Res. Soc. Symp. Proc. **519** (1998).

[4] S. Braun, S. Rappoport, R. Zusman, D. Avnir, M. Ottolenghi, *Mater. Lett.,* **10,** 1 (1990).

[5] C. Dave, B. Dunn, J.S. Valentine, J.I. Zink, *Anal. Chem.,* **66,** 1120A (1994).

[6] D. Avnir, S. Braun, O. Lev, M. Ottolenghi,*Chem. Mater.,* **6,** 1605 (1994).

[7] J. Livage, *C.R. Acad. Sci. Paris,* **322,** IIb, 417 (1996).

[8] C.J. Brinker, G.W. Scherer, in *Sol-Gel Science,* Academic Press, New York (1990).

[9] L.M. Ellerby, C.R. Nishida, F. Nishida, S.A. Yamanaka, B. Dunn, J.S. Valentine, J. Zink, *Science,* **25,** 1113 (1992).

[10] S.A. Yamanaka, F. Nishida, L.M. Ellerby, C.R. Nishida, B. Dunn, J.S. Valentine, J.I. Zink, *Chem. Mater.,* **4,** 495 (1992).

[11] Y. Tatsu, K. Yamashita, M. Yamaguchi, S. Yamamura, H. Yamamoto, S. Yoshikawa, *Chem. Letters,* 1615 (1992).

[12] P. Audebert, C. Demaille, C. Sanchez, *Chem. Mater.,* **5,** 911 (1993).

[13] S. Sampath, O. Lev, *Anal. Chem.,* **68,** 2015 (1996).

[14] J. Wang, D.S. Park, P.V.A. Pamidi, *J. Electroanal. Chem.,* **434,** 185 (1997).

[15] L. Bergogne, S. Fennouh, J. Livage, C. Roux, *Mat. Res. Soc. Symp. Proc.*. **519,** 171 (1998).

[16] M.T. Reetz, *Adv. Mater.,* **9,**943 (1997).

[17] A. Pandey, S. Benjamin, C.R. Soccol, P. Nigam, N. Krieger, V.T. Soccol, *Biotechnol. Appl. Biochem.* **29,** 119 (1999).

[18] in *Immobilized living cell systems,*Ed. R.G. Willaert, G.V. Baron, L. de Backer, John Wiley Pub. (1996)].

[19] C. Roux, J. Livage, K. Farhati, L. Monjour, *J. Sol-Gel Sci. Techn.,* **8,** 663 (1997).

[20] A. Turniansky, D. Avnir, A. Bronshtein, N. Aharonson, M. Altstein, *J. Sol-Gel Sci.Techn.,* **7,** 135 (1996).

[21] D. Shabat, F. Grynszpan, S. Saphier, A. Turniansky, D. Avnir, E. Keinan, *Chem. Mater.* **9,** 2258 (1997).

[22] J. Livage, C. Roux, J.M. Da Costa, I. Desportes, J.Y. Quinson, *J. Sol-Gel Sci. Techn.,* **7,** 45 (1996).

[23] P. Venkatesan, D. Wakelin, *Parasitology Today,* **9,** 228 (1993).

[24] L. Inama, S. Dire, G. Carturan, A. Cavazza, *J. Biotechn.* **30,** 197 (1993).

[25] E.J.A. Pope, K. Braun, C.M. Peterson, *J. Sol-Gel Sci. Techn.* **8,** 635 (1997).

[26] G. Carturan, G. Dellagiacoma, M. Rossi, R. Dal Monte, M. Muraca, *SPIE, Proceedings, Sol-gel optics IV,* **3136,**366 (1997).

[27] R. Campostrini, G. Carturan, R. Caniato, A. Piovan, R. Filippini, G. Innocenti, E.M. Cappelletti, *J. Sol-Gel Sci. Techn.,* **7,** 87 (1996).