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*REVIEW*

# THE USE OF SOL-GEL METHOD FOR BIOMATERIALS PREPARATION

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The paper refers to the field of biomaterials preparation by the sol-gel method. After the description of the process (general remarks and alkoxide, respectively aqueous routes presentation), its advantages are underlined. Further on, the study is dedicated to a special class of materials, respectively nanocomposites, emphasising the importance of bio-composites, especially of the enzymes-based ones. Some examples of sol-gel entrapped enzymes applications from the literature are given. The last part of the paper presents some original results regarding the preparation of enzymes-based biomaterials by the sol-gel method. It starts with the studies concerning the preparation of the amorphous silica matrices for enzymes encapsulation. Then, the results obtained entrapping two different classes of enzymes are presented. Glucose oxidase- and mixtures of proteases-based biomaterials with possible applications in the medical domain, respectively in the textile industry, have been obtained. All the presented compositions are original, none of them was taken from the literature.

## INTRODUCTION

### 1. Some general remarks regarding the sol-gel process

Considered as "unique" and "fascinating" both from scientific, and practical point of view,[1, 2] the sol-gel process has gained in the last years a great importance in the materials science field, being unanimously recognized for its advantages in preparing of some special materials and biomaterials with remarkable properties (electric, magnetic, optic, or of sensing, etc.).

Filho[3] defined the gelation process in 1988 as being the process which supposes the transformation of a sol into a wet gel. Four years later, Pierre[4] declared that practically there are so many definitions of the gelation process, as authors. He adopts however an alternative in which the idea of Filho can be recovered. Thus, he defines the gelation as a phenomenon through which a sol or a solution changes into a gel. The transformation supposes the establishment of some bonds either between the particles of the sol, or between the molecules of the solution, in order to form a final solid tri-dimensional network. But the situation is different from the classical solidification of a liquid, because in the case of the gelation, the solid structure remains opened and impregnated with the sol liquid or with the initial solution. This mixed composition "liquid-solid" confers to the gels some particular properties. One of the most accepted definitions of the sol-gel process belongs to Schmidt.[5] He considers that the essence of the process, at least in a first stage, consists from the synthesis of an inorganic amorphous network by a number of chemical reactions in solutions, at low temperatures. In a second stage, the inorganic network can be converted to a glass at temperatures much lower than the melting ones corresponding to the component oxides, or to a crystallized material at a temperature inferior to that used in the conventional methods. The essential stage of the process, respectively the transition from a liquid (solution or colloidal sol) to a solid (the di- or multi-phasic gel) led to the expression "sol-gel process", giving thus, practically, its name.

The definition given by Lopez and co-workers in 1990[6] is assumed by Ward and co-workers:[7] "sol-gel" is the name given to a great number of processes which suppose the existence of a solution or sol that turns into a gel.

No matter what definition is adopted it is evident that the gelation process means "sols" on the one hand and "gels" on the other hand. In such conditions an explanation of these terms is imposed. So, what are in fact, sols and gels? These constitute forms of manifestation of the matter which exist in natural state and which are known since the oldest times but only in the last period, beginning with the appearance and the development of the material sciences have started to present scientific interest.

The definition of a sol, from 1979, which belongs to Iler,[8] is assumed by Pierre[4] in 1992. In accordance with this, the sol represents a stable dispersion of (discrete, colloidal) particles in a liquid.

Zarzycki[9] considered in 1990 the wet gel as a material consisting from a solid skeleton impregnated with an interstitial liquid. In the same year, Hench and West[10] defined the gel as a rigid, interconnected network, presenting sub-micrometric pores and polymeric chains with medium length bigger than 1 μ. In 1992 Pierre[4] supplements this definition, specifying that the solid, tri-dimensional, interconnected network is developed in a stable manner inside of a liquid medium. The liquid which is present in the voids of the solid network is in thermo-dynamic equilibrium with the solid. According to the Science and Engineering Polymers Encyclopaedia, Almdal and co-workers[11] define the gel as a branched network of polymers which develops in a liquid medium, their properties being strongly dependent of interactions between the two components. The same authors quote a definition from the Webster dictionary, in accordance with which a gel is a gelatinous substance, formed from a colloidal solution that becomes a solid phase. It is the opposite of a sol. This last wording evidences the fact that the gel is a solid or semisolid material consisting of minimum two components, from which one is soft and elastic.

The condition that a sol becomes a gel (as a result of the gelation process) is that the sol passes through the so-called "gelation point". In 1988, Filho[3] pointed out that the definition of the gelation point in the literature is arbitrary and qualitative, without specifying some dynamic flow properties. The "gelation time" corresponding to the gelation point was qualitatively determined, at the room temperature, by visual inspection and was considered to be the time after which the sol does not cool under the gravity influence. Sakka and Kamiya[12] define the gelation point as the moment when the sol loses its fluidity and Yu and co-workers[13] consider it as the recorded time after which the surface of the sol remains unmodified at the inclination of the container during two minutes. Similar observations belong to Hench and West.[10] They specify that the gelation point, respectively the gelation time are easy to be observed from qualitative point of view and easy to be defined in abstract terms but very hard to be analytically measured. As the particles of the sol growth and collide together, the condensation and the formation of macro-particles take place. The sol becomes a gel when it can bear an elastic stress. This is the moment which literature defines typically as the gelation point (or time) of the sol. There is no an activation energy which could be measured and it could be not defined with precision the moment in which the sol turns from a viscous fluid into an elastic gel. The change takes place gradually according as more and more particles become interconnected. Some authors consider that the gelation of a silicatic solution is produced in the moment when the meniscus of the reaction mixture remains un-deformed at the inclination of the recipient.[14, 15]

Regarding the sol-gel transformation for the silica, although it is studied from long time ago, it is not completely elucidated till present. Pierre[4] underlined in 1992 the fact that although the silica gels exist in the nature as a manifestation of the matter since the oldest times, only at the end of the XX$^{th}$ century they were obtained by synthesis. After the colloids science appearance (whose founder is considered to be Graham, in 1861[4]), the publications about the "gelatinous" or "colloidal" forms have regularly increased, tending to elucidate their properties and structures. Beginning with 1970, the number of publications in the domain of gels and inorganic colloids increases exponentially.

The silica gels could be obtained by the sol-gel method using two different ways, respectively the alkoxide (organic) route and the aqueous (colloidal) one, which will be shortly presented in the next sections (2 and 3).

## 2. The alkoxide (organic) route of the sol-gel method

The alkoxide (organic) route represents, in fact, the most common way of synthesis (the so-called "classical") of the sol-gel process. As its name suggests, the precursors which are used in the synthesis consist from alkoxides (alcoolates). The precursors must be liquids able to form reactive monomers or oligomers. The compounds which could also be used as precursors (non-alkoxidic) consist from a series of aqueous solutions of some inorganic salts and/or from solutions of some organo-metallic compounds in the

corresponding solvents. These compounds assume a series of special problems which involve an apart approach even in the "organic" sol-gel domain. The most common and therefore the most used precursors remain the alkoxides. Their simplified chemical formula is $M(OR)_n$, which indicates the fact that they represent the result of a direct chemical reaction between a metal and an alcohol.[4] Their developed chemical formulas are not always known. Anyway, in all cases they suppose M-O bonds, respectively "metal-oxygen". In the indicated general formula "M" represents a metal (Si, Ti, Al, etc.) and "R" indicates an organic radical as methyl, ethyl, propyl, buthyl or another alkylic group. These organic groups are very important because they are the only ones which could introduce a certain degree of contamination, they must confer enough stability and volatility to the alkoxide in order to allow its manipulation and must yield to the net breaking of the M-OR or MO-R bonds enough early during the chemical process in order to form, finally, an inorganic, pure polymer based on M-O-M type bonds. In the case of alkoxides, the degree of ionicity of the M-O bond, which depends on the size and on the electro-negativity of the metallic atom, is also very important.

The chemical elements which can realize the final product by mean of the sol-gel process must be hydrolizable that is to allow the construction of a complex scale of molecules containing O or OH groups as ligands, condition which is fulfilled by the alkoxides. Normally they dissolve into an alcohol and hydrolyse when water is added, in acid or alkaline conditions. The role of hydrolysis consists just in the transformation of the alkoxide-type ligand into hydroxyl-type one. After that, further condensation reactions will lead to the obtaining of M-O-M, respectively M-($_\mu$OH)-M (where $_\mu$OH refers to hydroxy bound groups) based polymers.[16] The chemistry of alkoxides hydrolysis and polymerization is very complex. The chemical reactions which take place are:[4]

- hydrolysis reactions:

$$M(OR)_n + x\ H_2O \rightarrow M(OH)_n(OR)_{n-x} + x\ ROH \quad (1)$$

- polymerization-condensation reactions through dehydration:

$$\text{-M-OH + HO-M-} \rightarrow \text{-M-O-M-} + H_2O \quad (2)$$

- polymerization-condensation reactions through alcohol elimination:

$$\text{-M-OH + RO-M-} \rightarrow \text{-M-O-M-} + ROH \quad (3)$$

- polymerization-condensation reactions through ether elimination:

$$\text{-M-OR + RO-M-} \rightarrow \text{-M-O-M-} + ROR \quad (4)$$

All these reactions take place in a solvent which is generally the corresponding alcohol. Moreover, they are often reversible. Depending on the relative kinetics of the condensation and hydrolysis reactions it is possible to result either linear polymers or dense colloidal particles or intermediate colloidal particles consisting from "balls" of weak reticulate polymers. These structures could be confirmed by the SAXS method (Small Angle X-ray Diffraction) or by viscosity measurements.[4]

It is considered that a slow hydrolysis reported to condensation in the case when the polymerization products do not re-dissolve favours the formation of linear polymer structures and leads to the obtaining of polymeric gels. This polymerization can be catalyzed by acids or alkalis. The weak hydrolysis can be due to some chemical characteristics of the alkoxide or can be artificially produced for example by choosing a hydrolysis water quantity enough for the imposed stoichiometry of the alkoxide. It is also possible a second alternative in which the hydrolysis is rapid, the polymerization-condensation reactions being weaker or being possible to be revoked by re-dissolution. In this case either hydrate colloidal oxides (when the condensation products re-dissolve) or aggregates of hydrolyzed monomers as a result of reactions (5) and (6) could be obtained.[4]

$$M(OR)_n + n\ H_2O \rightarrow M(OH)_n + n\ ROH \quad (5)$$

$$M(OH)_n \rightarrow MO_{n/2} + n/2\ H_2O \quad (6)$$

The promoting of hydrolysis in a first step by using an excess of water facilitates the obtaining of relative massive monoliths, the polymers network being reticulate in this case.

The chemistry of alkoxides was profoundly studied for the silicium, in which case, depending on the adopted chemical protocol, different types of silica gels could be obtained.[4]

The silica gels result from the hydrolysis (reactions (7)-(10)) and poly-condensation (reactions (11)-(12)) reactions of the Si alkoxides:[17]

$$Si(OR)_4 + H_2O \leftrightarrows HO\text{-}Si(OR)_3 + ROH \quad (7)$$

$$HO\text{-}Si(OR)_3 + H_2O \leftrightarrows (HO)_2\text{-}Si(OR)_2 + ROH \quad (8)$$

$$(HO)_2\text{-}Si(OR)_2 + H_2O \leftrightarrows (HO)_3\text{-}Si\text{-}OR + ROH \quad (9)$$

$$(HO)_3\text{-}Si\text{-}OR + H_2O \leftrightarrows Si(OH)_4 + ROH \quad (10)$$

$$\equiv Si\text{-}OR + HO\text{-}Si\equiv \leftrightarrows \equiv Si\text{-}O\text{-}Si\equiv + ROH \quad (11)$$

$$\equiv Si\text{-}OH + HO\text{-}Si\equiv \leftrightarrows \equiv Si\text{-}O\text{-}Si\equiv + H_2O \quad (12)$$

In the case of an acid catalysis (pH < 2.5) depending on the proportion of the hydrolysis water it is possible to obtain either high linear species or accidentally branched out or even "balls" of colloidal branched out polymers. In all these cases the hydrolysis controls the process related to polymerization and the reaction rate is proportional with the $[H_3O]^+$ ions concentration, that is with the result of $[H^+]$ and $[H_2O]$ concentrations multiplication. In the case of an alkali-catalysis (considered in the literature for pH > 2.5) the condensation is accelerated and the kinetic is proportional with the $[OH]^-$ ions concentration. In this case either dispersed colloidal silica particles (at higher pH values) or dense "balls" of polymers which branches ensure a partially, weak connexion between them could be obtained.

## 3. The aqueous (colloidal) route of the sol-gel method

The aqueous (colloidal) route of the sol-gel method, as the name suggests, uses as precursors aqueous or colloidal solutions. The most common are the silicic acids solutions from different sources: either one treats an aqueous solution of an alkaline silicate with a mineral acid or replaces the alkaline ions ($M^+$) with $H^+$ ions by passing the solution through an ion-exchange column or a colloidal silica sol is de-stabilized. No matter the choosed alternative the result will be the same: the obtaining of silicic acids solutions which by polymerization lead to silica gels. In fact, the monomer silicic acid $Si(OH)_4$ was never possible to be isolated.[8] It is a very weak acid which can not exist but in diluted aqueous solutions, its concentration leading immediately to the polymerization. Being a non-ionic substance, neutral, and strongly hydrophilic, the mono-silicic acid can not be separated from the water.

A totally remarkable scientific contribution regarding the sol-gel process study of the aqueous silica belongs to Iler.[8] He studied the aggregation of colloidal particles, considering that this process comprises all possibilities of union between them. But he makes the distinction between the gelation and the other types of aggregation, as precipitation, coagulation and/or floculation. Iler defines as gelation process only the situation in which the particles join each other forming branched chains which occupy the whole volume of a silica sol so that none increased silica concentration in any macroscopic region of the medium could be observed. Instead, the whole medium becomes viscous and then solidifies into a rigid network of particles which retains the liquid through a capillary action. Moreover, the refraction index of the micro-gel is the same with that of the environing medium, thus the micro-gel can not be seen and the density remains also un-modified, which explains the fact that the micro-gel doesn't depose even by centrifugation. Any type of aggregation which doesn't respect these conditions has not as result the formation of a gel but of a precipitate or coagulate or of some flocons.

In 1979, Iler[8] described the formation of silica gels as a polymerization process consisted from three stages. His theory remained valid until our days. The three stages are:

a) the monomer polymerization, in order to form the particles;

b) the growth of the particles;

c) the joint of the particles in order to form branched chains, then networks which will extend finally in the whole liquid medium, "thickening" it as a gel.

It is important to note that all real silicatic solutions (aqueous colloidal silica sols and aqueous solutions of alkaline silicates) present from the beginning a certain polymerization degree. Because of this reason the study of their gelation practically starts from the second stage above mentioned.

In the case of the aqueous route of the sol-gel method the chemistry of the process is also very complex (as in the alkoxide route case). It consists from a combination of hydrolysis-polycondensation reactions. The process is controlled, essentially, by two types of reaction equilibria: acid-alkali, corresponding to reaction (13), and polymerization – de-polymerization, corresponding to reaction (14):[18]

$$\equiv Si\text{-}OH + H_2O \leftrightarrows \equiv Si\text{-}O^- + H_3O^+ \quad (13)$$

$$\equiv Si\text{-}OH + HO\text{-}Si\equiv \leftrightarrows \equiv Si\text{-}O\text{-}Si\equiv + H_2O \quad (14)$$

The polymerization – de-polymerization equilibrium can be represented not only by the elementary condensation of the neutral silicic acid molecules (reaction (14)) but also by an effective chemical reaction between the ionized and non-ionized acids (see reaction (15)).[19]

$$\equiv Si\text{-}OH + {}^-O\text{-}Si\equiv \leftrightarrows \equiv Si\text{-}O\text{-}Si\equiv + HO^- \quad (15)$$

Andersson and co-workers[18] have studied the relation polymerization – de-polymerization and the colloids and gels formation in a series of silicatic solutions both in acidic and alkaline domain. They have followed the changes that occur using a large variety of techniques as: pH measurements, trimethylsililation, classical and dynamic light diffusion, ultra-filtration, and $Si^{29}$ RMN spectroscopy. The conclusion of the study was that both for acidic and alkaline solutions there is no a direct correlation between the rate of monomer disappearance and the gelation rate. There is no a real evidence of the fact that both processes (polymerization and gelation) are directly correlated, belonging to a continuous process analogous to a typical organic polymerization. It is more probably that the process can be divided in some stages. Thus the authors[18] take into consideration and practically confirm the three stages of silica gel formation mentioned by Iler.[8] Later, Beelen and co-workers[20] confirm also this approach. They consider that the polymerization or particle formation and the aggregation are not strictly successive processes. They are both based on the same reaction (the condensation) so they are not rival processes.

In the case when the precursor is an aqueous solution of an alkaline silicate instead of an aqueous colloidal silica sol the situation is more complicated. The distribution of silicatic species in the solution is dependent, among other things, by the nature of the used alkaline cation.[21, 22] Studying the effect of the alkaline metals on the aqueous silicatic systems in which they are present, the authors suppose that the silicatic ions combine with alkaline cations as ion-pairs. The high oligomers suffer a preferentially stabilization by the alkaline cations which are more heavy. Iler considers[23] too that in the alkaline silicate solutions all $M^+$ ions are combined both with the monomer and with the oligomers and with the colloidal particles as ion-pairs. The concentration of alkaline cations can significantly affect the structure of the solution. Kinrade and Pole[24] explain this by the stabilization of the oligomers. They suppose a reaction mechanism in which the cations intercede the condensation of silicates by diminishing the repulsive coulombien forces between the anions (reactions (16) and (17)).

$$(HO)_3\equiv Si\text{-}O^- + M^+\ldots O^-\text{-}Si\equiv(OH)_3 \leftrightarrows (HO)_3\equiv Si\text{-}O^-\ldots M^+\ldots O^-\text{-}Si\equiv(OH)_3 \quad (16)$$

$$(HO)_3\equiv Si\text{-}O^-\ldots M^+\ldots O^-\text{-}Si\equiv(OH)_3 \leftrightarrows {}^-(HO)_2\equiv Si\text{-}O\text{-}Si\equiv(OH)_3 + MOH \quad (17)$$

The above mentioned three stages of the gelation process which lead to the formation of a gel in the case of colloidal silica sols remain valid for the gelation of alkaline silicate solution too. The silica particles initial formed can grow until colloidal dimensions (generally 1-100 nm in diameter).[25] These primary particles are unstable reported to aggregation because in aqueous colloidal solutions of alkaline silicates the double electric layer formed by ionization of the silanol groups of the surface is suppressed by the adsorption of the opposite sign ions present in system. In the second stage of the process, the aggregation of the primary particles, it is possible to note a certain stability against the aggregation, because of the hydration forces of the water molecules which surround the silica particles. According as the gelation proceeds the structure modifies. The statistic analysis[25] prove that the growth of chains is unlimited, being necessary to form the rings. The HMO calculus (Huckel Molecular Orbital Theory) and the INDO one (Intermediate Neglect of differential Overlap Molecular Theory) show that the "chain" structures are more stable than the "rings" in the case when 3-4 (according to the HMO theory), respectively 10-12 (INDO) silica tetrahedra are involved. The elimination of the difference between the molecular energies of the chains and rings seems to limit the growth of the primary particles to rings containing approximately 5 tetrahedra per particle.

As in the case of the alkoxide route of the sol-gel method, in the aqueous one it is possible to control the process parameters (pH, temperature, concentrations) such as to obtain gels with certain structural properties.

### 4. The advantages of the sol-gel process as synthesis method

The sol-gel process represents an exceptional synthesis method, its advantages being those which sustain and recommend it.[4, 5, 26, 27] Essentially, they are:

- the chemical process takes place at low temperature, at least in a first stage, which minimizes the chemical interactions with the walls of the recipient, that is different from the case of the synthesises at high temperatures;
- the kinetic of the different chemical reactions can easy be controlled because of the low temperatures and of the frequent dilution conditions which is considered one of the major advantage of the sol-gel method;
- the association of both colloidal state of the solid material and the liquid one automatically lead to the elimination of pollution by the dispersing of powder;
- the final reaction products obtained with purified precursors (by distillation, crystallization or electrolytic) can be very pure;
- it allows the control of the nucleation and growth of primary colloidal particles in order to obtain certain shapes, sizes, and size distributions in a sub-microscopic domain; the sol-gel process guarantees the possibility to obtain nano-metric reaction products which represents one of the most special achievement of the sol-gel method;
- it is possible to control not only the size of the particle but the structure of the sol-gel product also, the synthesis being able to produce amorphous, semi-vitreous and/or vitreous materials; the fact that the structure can be pre-determined by the agency of experimental conditions it's very important and confers the superiority to the sol-gel process compared with any other synthesis method;
- depending on the experimental conditions of the process different forms of the final reaction products could be obtained: monoliths, powders, fibres, wires, coatings;
- it allows to obtain not only any oxide-based composition but very special reaction products also which are of great and present-day interest, as:

a) complex systems of mixed oxides whose homogeneity can be controlled until atomic level;

b) inorganic-organic hybrid materials obtained by introducing some organic permanent groups.

## COMPOSITES OBTAINED USING THE SOL-GEL PROCESS (NANOCOMPOSITES)

### 1. The definition of nanocomposites

The term of "nanocomposite" was introduced by Roy, Komarneni, and co-workers,[28] during the period 1982-1983, in order to evidence the major conceptual re-direction of the sol-gel process in order to use it for create maximally heterogeneous rather than homogeneous materials. They realized di- and multi-phasic nano-heterogeneous sol-gel materials. According to the mentioned authors, "nanocomposite" materials should be clearly differentiated from the "nanocrystalline" and "nanophase" ones, which refer to single phases in the nanometre range. "Nanocomposites" refers to composites of more than one Gibbsian solid phase where at least one dimension is in the nanometre range and typically all solids phases are in the 1-20 nm range. The solid phases can be amorphous, semi-crystalline or crystalline or combinations thereof. They can be inorganic, organic, or any combination of both and can have any chemical composition.

### 2. The classification of nanocomposites

Based on their material function, physical and chemical differences, and temperature of formation, Komarneni[28] has realized a classification of nanocomposites in five major groups:

1) sol-gel nanocomposites, which are obtained at low temperatures (< 100°C); after the thermal treatment at high temperature it is possible to obtain homogeneous mono-crystalline ceramic phases or multi-phasic crystalline ceramics;

2) intercalation-type nanocomposites, which can be prepared at low temperatures (< 200°C) and lead to useful materials after heating to modest temperatures (< 500°C);

3) entrapment-type nanocomposites, which can be obtained from three-dimensional structures linked network structures such as zeolites which can also be synthesised at low temperatures (< 250°C);

4) electroceramic nanocomposites, which can be prepared by mixing nanophases of ferroelectric, dielectric, superconducting, and ferroic materials in a polymer matrix at low temperatures (< 200°C);

5) structural ceramic nanocomposites, which are obtained by traditional ceramic processing at very high temperatures (1000-1800°C).

Komarneni[28] subdivides each of these five major groups of nanocomposites and describes them in detail. Regarding the sol-gel nanocomposites, they are subdivided in six categories:

a) compositionally different sol-gel nanocomposites - these represent very intimate mixtures composed of two or more solid phases that differ in composition and each have particle sizes of order of 10-20 nm. Solid phases of these dimensions produce "sols" when dispersed in a liquid. Two or more sols of different compositions can be uniformly mixed and gelled in order to obtain compositionally different nanocomposites (e.g., $Al_2O_3$-$SiO_2$, $SiO_2$- MgO, $Al_2O_3$-$TiO_2$, etc.);

b) structurally different sol-gel nanocomposites - these consist of two or more solid phases with the same composition but different structures. Examples include mixtures of ultra-fine crystalline seeds in amorphous or semi-crystalline xerogels, as $\alpha$-$Al_2O_3$ seeds in $Al_2O_3$ gels or $TiO_2$ films on single-crystal substrates;

c) both compositionally and structurally different sol-gel nanocomposites - these represent a combination of the above two types of nanocomposites and consist of compositionally discrete phases with crystalline seeds of the equilibrium phase. Examples include following combinations: $ZrO_2$-$SiO_2$, $ThO_2$-$SiO_2$, $Al_2O_3$-MgO;

d) nanocomposites of gels with precipitated phases - these are one type of ceramic-ceramic nanocomposites which are prepared by the growth of extremely fine crystalline or non-crystalline phases inside the pores of a pre-made gel (e.g., $SiO_2$) structure. The growth of the fine phases is accomplished by soaking the gel in a metal salt solution and subsequent precipitation of the metal with selected anions. Examples include photocromic glasses and catalytic materials;

e) nanocomposites of xerogels with metal phases - In this situation the sol-gel process was extended to the preparation of new di-phasic xerogels leading to new ceramic-metal nanocomposite materials. As examples could be mentioned the prepared $Al_2O_3$, $SiO_2$ and $ZrO_2$ xerogels that were matrices for 5-50 nm dispersed metallic phases consisting in Cu, Pt, and Ni. Very finely dispersed metal particles (2-4 nm) have been deposited by liquid- and gas-deposition techniques in sol-gel membranes;

f) nanocomposites of inorganic gels and organic molecules (dyes) - The sol-gel process allows the incorporation of optically active organic molecules in the porous gel or in glass-like matrices because the gels can be prepared at room temperature and their porosity can be controlled. One can incorporate the organic species including polymers during gelation or the organic molecules can be introduced into the pre-made sol-gel matrices through diffusion. Different types of laser dyes, conducting and conjugated polymers and/or polymers containing hydrogen-bond acceptor groups and photochromic molecules have been successfully incorporated into silica gels. The resulted nanocomposites may have interesting optical, non-linear optical, conducting and/or photochromic properties with potential applications in optical devices, chemical sensors and laser materials. Further studies in this domain refer to the interactions between the sol-gel matrix and the guest molecules in order to optimize the properties of the resulted nanocomposites for various applications.

## 3. The importance of nanocomposites

Nanocomposites represent a category of relatively new materials (from the last 20 years),[28] very actual, that arouse an exquisite interest. This fact is due to the special properties which the nanometric particles confer to the materials, being well known the dependence of the physical properties of a material of the dimension of its particles.[29] In this moment, the obtaining of the nanocomposites by the sol-gel method is well accepted for catalytic, optic and electro-ceramic materials as well as for sensors. Today one can speak about "nanotechnologies", whose essence consists in the skill to operate at molecular level, atom by atom, in order to create big structures with a fundamentally modified organization. They refer to materials and

systems whose structures and components present new and significantly improved physical, chemical, and biological properties, because of their nanometric dimensions. In the present there is a preoccupation regarding the exploitation of these improved properties (electric, magnetic, optic, catalytic, biologic, etc.) by studies which can guarantee the control of the respective structures and the achievement of functional devices (e.g., sensors). That is why the impact of the nanotechnologies is huge, being widespread in the majority of the most important domains, as: the preparation of materials, the computers technology, the medicine and the health, aeronautics, the ambient medium, the biotechnology and the agriculture.

## BIOCOMPOSITES

### 1. What the biocomposites are?

The interest for the biomaterials domain is justified by the importance of this type of materials in numerous domains, as: medicine, food industry, textile industry, detergents, agriculture.

In 1984, Avnir and co-workers[30] referred to a veritable "revolution" in the chemistry and physics of materials. A major and inherent difficulty which hindered the combination of the organic chemistry with the glass and ceramic chemistry was surpassed. The responsible parameter for the incompatibility of the two domains (the organic chemistry, on the one hand and the glass and ceramic chemistry on the other hand) was the temperature, being well known the fact that there are very few organic compounds which can resist at temperatures over 200°C (and bio-molecules resist even at temperatures much lower than 200°C), while in the case of glasses and ceramics the usual values of this parameter are currently over 1000°C. The process which solved this problem was the sol-gel one. Because it takes place at low temperature (the room temperature), the sol-gel method allows the entrapment in the porous network of the resulted gel of some molecules which are less stable from chemical and thermic point of view, as bio-molecules (proteins, enzymes, antibodies, bacteria). The doped bio-molecules from the porous silica gel network become a component part of its nanostructured architecture. The matrix gel retains and maintains the native conformation of the bio-molecule, being at the same time enough rigid from physical point of view, enough inert from chemical point of view and stable from thermic point of view to allow the performing of the experiences. Moreover, the host matrix offers to the bio-molecule the necessary protection against the microbial attack. All these conditions are accomplished by the silica gels, which are, moreover, transparent too, property that represent a supplementary trump, making them unique for the realization of fluorescent, luminescent and/or colorimetric sensors. The use of $SiO_2$ gels as matrices for different bio-compounds is well established in the present moment.[31-69] Today one can speak about a new class of hybrid inorganic-organic materials, respectively the "sol-gel bio-materials" or "bio-composites" which present the chemical and bio-chemical characteristics of the included bio-component. They can be framed in the "nanocomposites of inorganic gels and organic molecules" category which is a sub-class of the "sol-gel nanocomposites" from the classification of Komarneni.[28] The flexibility of the chemistry of the sol-gel process allows the entrapment of almost any type of bio-molecule in the silica matrix that is why the "list" of sol-gel bio-materials, respectively of bio-composites is in a continuous increase.

### 2. Enzymes-based biocomposites

The enzyme-based biocomposites represent the most studied sub-category of this type of materials. This is due to their numerous applications in very important domains, as: medicine, textile industry, detergents, catalysis.

It is well known that enzymes are biocatalysts (natural catalysts). The considerable importance granted to the enzymatic research domain is justified by the spectacular results obtained in the enzymatic catalysis compared to the classical one. When a reaction can be catalyzed either by an enzyme or by simple substances (acids, alkali or different metallic ions) it can be observed that the enzymatic reaction devolves with a much higher rate, so its activation energy is much lower. A supplementary advantage of the enzyme use consists in the infinitesimal quantities required in order to obtain considerable effects, because of the especial catalytic activity. Instead, compared to the classic catalysts, the enzymes present the drawback of the vulnerability.

They can denaturate (they loose their tri-dimensional structure) relatively easy in conditions of excessive heating or at extreme values of the pH or in the presence of some chemical agents. All these difficulties can be solved by the sol-gel process, which immobilizes the enzymes in a protective matrix that is the silica gel. An immobilization method is considered advantageous if it fulfils the following conditions:[39]

- is simple and rapid;
- is non-specific;
- the immobilized compound is stable and don't leave the host matrix;
- the immobilized compound maintains its chemical and bio-chemical activity.

There are three immobilization methods which are the most used:[39, 40]

1) the adsorption onto a solid substrate (physical adsorption);

2) the covalent binding;

3) the entrapment (encapsulation) in a porous, tri-dimensional network.

1) The adsorption onto a solid substrate is considered to be the most simple immobilization method. However, in the case when bio-compounds are immobilized this method presents a major inconvenient. It consists from the possibility to miss them by desorption from the support in the absence of some covalent bonds. In this case a decrease of the response in time, respectively a too short effective "life-time" of the material can be observed.

2) The covalent binding method supposes the realization of a specific, chemical bond between the immobilized compound and the substrate. Because of this bond, the probability to "lose" the compound, as in the precedent method is much lowered so the "life-time" of the material increases significantly. Instead, when the bio-compounds have to be immobilized, the possible chemical reactions can disturb till to the destruction of the bio-molecule. Moreover, the method supposes the use as substrate of the modified silica (by silanization or with glutaraldehide), which implies two difficulties: a higher cost, and a bigger time consumption.

3) In the entrapment (encapsulation) method, the compound is physically captured in the porous, tri-dimensional matrix from which it can't emerge (or goes out very hard). This immobilization method avoids the drawbacks and combines the advantages of methods "1" and "2", as it can also be seen from Table 1.

Table 1 presents a comparative study regarding the characteristics of the above mentioned immobilization methods.[31]

*Table 1*

Comparison of the attributes of different classes of immobilization techniques

| Characteristic | Physical adsorption | Covalent binding | Entrapping |
|---|---|---|---|
| Preparation | Simple | Difficult | Difficult |
| Binding force | Weak | Strong | Intermediate |
| Enzyme activity | Intermediate | High | Low |
| Regeneration of carrier | Possible | Rare | Impossible |
| Cost of immobilization | Low | High | Intermediate |
| Stability | Low | High | High |
| General applicability | Yes | No | Yes |
| Protection of enzyme from microbial attack | No | No | Yes |

The immobilization of an enzyme can modify both the reaction kinetics and some properties of the enzyme, being often observed a decrease of the specific activity. The modification of the enzymatic properties is due to some factors, as:[31]

a) conformational effects - when, because of some amino-acid modifications in the active centre of the enzyme, one can observe some changes in the conformation of the enzyme molecule; the charge of the enzyme can also be modified;

b) steric effects - when the interaction of the substrate with the enzyme is affected by the steric hindrance;

c) partitioning effects - related to the chemical nature of the support material; they can appear as a result of the electrostatic or hydrophobic interactions between the matrix and low molecular species present in the solution, leading to a modified microenvironment;

d) mass transfer or diffusional effects - which arise from the diffusional resistance to the transport of substrate from the bulk solution to the catalytic active sites and from the diffusion of products of the reaction back to the bulk solution.

The observed changes regarding the enzymatic properties in the case of immobilization represent the result of the interactions between all these factors, being very hard to establish the accurate effect of each one of theme.

Because of the conformation modifications, steric hindrance, partition and diffusion effects, which take place either simultaneously or separately, in the case of the immobilized enzymes the kinetic constants are most frequently different from those corresponding to the enzymes in their native form.

A simple chemical reaction typically presents a dependence of reaction rate on reactant concentration, thus the increasing of the concentration leads to a non-limited increase of the rate. In an enzymatic catalyzed reaction, the rate also grows with the substrate concentration increase, but in a non-definite manner. It tends to a limit, when it can be considered that the enzyme becomes saturated. The simplest explanation of this behaviour is due to so-called "Michaelis-Menten mechanism" from which the study of the enzymatic catalyzed reactions starts.[31] This supposes that an enzymatic catalyzed reaction involves some stages. First, the enzyme (E) reacts with the substrate (A), resulting an enzyme-substrate complex (EA). This is a rapid reaction. Subsequently, a lent conversion of the complex in the reaction product (P) takes place, P being liberated in the same time with the free enzyme, regenerated in its initial form:

$$E + A \leftrightarrows EA \rightarrow E + P \tag{18}$$

The mathematic expression of the Michaelis-Menten equation is:

$$v = (k_0 \cdot e_0 \cdot a)/(K_m + a) \tag{1}$$

where: v = reaction rate;
a = substrate concentration;
$e_0$ = total concentration of the enzyme;
$k_0$ = catalytic constant;
$K_m$ = Michaelis constant.

Briggs and Haldan[31] have re-write the Michaelis-Menten mechanism (reproduced by equation (1)) thus:

$$E + A \underset{k_{-1}}{\overset{k_{+1}}{\rightleftharpoons}} EA \underset{k_{-2}}{\overset{k_{+2}}{\rightleftharpoons}} E + P \tag{19}$$

They consider that no matter the values of the rate constants in different stages ($k_{+1}$, $k_{-1}$, $k_{+2}$, $k_{-2}$), initially it can be observed a rapid increase of the EA (intermediary complex) concentration but after than it passes through an equilibrium state because it is converted into the reaction product as rapidly as it is formed. This supposition is known as the equilibrium state theory and lies at the basis of approximately all kinetic studies of the enzymes. In this variant, the Michaelis-Menten equation [equation (1)] becomes (2):

$$v = \frac{k_{+2} \cdot e_0 \cdot a}{[(k_{-1} + k_{+2}) / k_{+1}] + a} \tag{2}$$

which is of the same form, differing by the parameters significance, that is: $k_0 = k_{+2}$

$K_m = (k_{+1} + k_{+2}) / k_{+1}$

The Michaelis constant ($K_m$) is a very important kinetic parameter which determination is necessary because it reflects the existing affinity between the substrate and the enzyme. In the case of an immobilized enzyme this parameter can change. $K_m$ can decrease, that indicates a faster rate of reaction than in the case of the free enzyme. $K_m$ can also decrease if the charges on the support and substrate are opposite. This diminution can be attributed to partition effects, due to an increase of the electrostatic attractive forces

between the carrier and the substrate, which lead to an increase in the concentration of the substrate in the microenvironment of the immobilized enzyme. Another situation in which the $K_m$ value can diminish is that in which the internal and external diffusion effects interfere, if the enzyme particle is surrounded by an un-stirred layer (Nernst layer) of solvent in which the concentration of substrate is lower that in the bulk solution. This effect can be reduced either by diminishing the particle sizes of the immobilized enzyme or by increasing the linear rate of the fluid. It must be mentioned the special importance of the internal diffusion effects in the case when the enzyme is immobilized in a porous support (matrix) which is the case of the entrapment techniques.

The Michaelis constant can also have higher values because of the immobilization of the enzyme. This assumes the use of a higher substrate concentration in order to reach the same reaction rate as in the case of the free enzyme. The conformational changes of the enzyme protein molecule and the steric hindrances usually lead to the increase of the $K_m$ value, because of the diminution of the affinity between the enzyme and the substrate. These effects are more pronounced in covalent binding techniques in which the enzyme molecule is chemically modified.

No matter the immobilization bio-catalytic technique used, and the catalytic process involved compared to the use of free enzymes the superiority of the first mentioned alternative is evident. This is clearly expressed by the Table 1, which presents the principal design factors of bio-reactors in both situations.[31]

Table 2 can be considered as a synthesis of arguments of the advocates of the immobilized biocatalysts alternative. In fact, in the present moment the importance of the immobilized enzymes in order to improve their stability is well recognized.[31, 35, 38, 39, 43-46, 70-75]

*Table 2*

Comparison of soluble and immobilized bio-catalysts

| Crt. no. | Design factor | Soluble bio-catalyst | Immobilized bio-catalyst |
|---|---|---|---|
| 1. | Separation from product | Not feasible (denaturation possible *e.g.*, by heat) | Easy (being possible to reuse the bio-catalyst) |
| 2. | Continuous operation | Not feasible | As a rule |
| 3. | Reaction time | Long (low enzyme concentration to minimize enzyme cost) | Short |
| 4. | Inhibition effects | Cannot be controlled | Can be controlled by a proper choice of reactor |
| 5. | Reactor size | Relatively large | Relatively small |
| 6. | Influence of mass transfer | None | Usually strong |
| 7. | Stability | Usually less stable in solution | Enhanced (majority of cases) |
| 8. | Specific activity per unit volume of reactor | Could be very high (usually not economical) | Much less than for soluble enzymes (generally by a factor of 100) |
| 9. | Reaction control/ automation | Often not feasible | Easy |
| 10. | Contamination of product | By enzyme | None |
| 11. | Complexity of operation | Very simple | Usually more complex |

## 3. The advantages to adopt the sol-gel process for bio-composites preparation

Even the general advantages of the sol-gel process as a synthesis method have already been enumerated (see section 1.4.), it is necessary to underline the aspects related of its use for the peculiar case of biomaterials preparation, respectively:

- the chemical process takes place at low temperature (room temperature);
- the porous network of the resulted gel can encapsulate different bio-molecules (proteins, enzymes, antibodies, bacteria), with low thermal and chemical stability;
- the porosity of the silica gel matrix ensures a large surface area;
- the gel retains and maintains the native conformation of the bio-molecule;
- the doped bio-molecules are fixed in the porous network of the gel as component part of its nanostructured architecture;
- the protective role of the matrix consists in ensuring the mechanic resistance, the chemical inertia, the thermal stability, and the protection against the microbial attack.

As a peculiar case of the biomaterials the example of sol-gel immobilized enzymes can be given. In this situation, Kennedy and co-workers[31] refer to the following advantages:

a) enzymes can be reused;
b) processes can be operated continuously and can be readily controlled;
c) products are easily separated;
d) effluent problems and materials handling are minimized;
e) in some cases, enzyme properties (activity and stability) can be altered favourably by immobilization.

## 4. Applications of sol-gel entrapped enzymes

Since 1987 Kennedy[31] considered that enzymes are the focal point of the biotechnological processes, and that without them, biotechnology as a subject would not exist. He and co-workers have intensively studied the applications of either free or immobilized enzymes. They refer to four major domains of applications:

1) biocatalyst reaction engineering;
2) food and feed processing;
3) pharmaceutical and chemical industries;
4) analytical applications of enzymes: enzyme sensors for clinical, process, and environmental analyses.

All these domains remain valid concerning the applications of sol-gel entrapped enzymes. In his excellent recent review regarding the sol-gel encapsulation of enzymes Pierre[67] presents a list of sol-gel enzyme-based biosensors. The list is based on 29 references and refers only to a very short period (2001-2003). Table 3 contains the mentioned data.

*Table 3*

List of biosensors with gel encapsulated enzymes recently studied

| *Enzyme* | *Analyte* | *Immobilization medium* | *Sensor type* |
|---|---|---|---|
| Glucose oxidase, Catalase + dyes | Glucose | $SiO_2$ gel on inner surface of a glass tube | Luminescence |
| Trypsin | Proteins | $SiO_2$ gel in microcolumns, microchannels, and microarrays | Mass spectrometry |
| Alkaline phosphatase | Pesticides | $SiO_2$ gel | Fluorescence |
| Urease + fluorescent dye | Heavy metals | $SiO_2$ gel in well strip | Fluorescence |
| Alcohol oxidase | Alcohol | $SiO_2$ gel | Colorimetric |
| NADH: FMN-oxidoreductase-luciferase | Quinones and phenols | Starch gel | Luminescence |
| Horseradish peroxidase + luminol and uricase | Uric acid | $SiO_2$ gel in a microreactor coupled to chip and a microfluidic system | Luminescence |
| Glutamate deshydrogenase + thionine | Glutamate | $SiO_2$ gel on tip of fibre optic | Fluorescence |
| Hydrophilic enzyme (urease) | Urea | Free or dextran conjugated fluorescence dye + silica gel, cast film | Fluorescence |
| Lipophilic enzyme (lipase) | Glyceryl tributyrate | Free or dextran conjugated fluorescence dye + silica gel, cast film | Fluorescence |
| Cholinesterase | Pesticides | Three layer sandwich membrane<br>- Enzyme in hydrophilic modified<br>- Polyvinylidene-fluoride bromcrome sol purple in intermediate sol-gel membrane | Absorbance |
| Glucose oxidase | Glucose | $SiO_2$ gel on oxygen electrode | Amperometric |
| Glucose oxidase + bovine serum albumin | Glucose | Hybrid alumina gel + polyphenol membrane | Amperometric |
| Glucose oxidase | Glucose | $SiO_2$ gel on oxygen electrode | Amperometric |
| Glucose oxidase, polyphenol oxidase, or horseradish peroxidase | Glucose, phenol, catechol $H_2O_2$ | $TiO_2$ gel + vinylpyridine-grafted polyvinyl alcohol solution | Amperometric |
| Invertase, lactase and maltase with glucose oxidase | Disaccharides | $SiO_2$ gel on oxygen electrode | Amperometric |
| Aldehyde dehydrogenase | Acetaldehyde, wines | $SiO_2$ gel on screen-printed carbon | Amperometric |
| Horseradish peroxidase | Phenol | Adsorption on $SiO_2/Nb_2O_5$ gel + glutaraldehyde crosslinking + mixing with graphite powder | Amperometric |

*Table 3* (continues)

*Table 3* (continued)

| | | | |
|---|---|---|---|
| Invertase, lactase and maltase with glucose oxidase | Disaccharides | $SiO_2$ gel on oxigen electrode | Amperometric |
| Urease | Heavy-metal ions | $SiO_2$ gel on thick film electrode | Amperometric |
| Acethyl-cholinesterase (AChE) | Pesticides | $SiO_2$ gel coating on screen-printed electrode | Amperometric |
| Acethyl-cholinesterase (AChE) | Pesticides | Photopolymerizable polymer coating on screen-printed electrode | Amperometric |
| Cholesterol oxidase | Cholesterol | $SiO_2$ gel + poly(tetrafluoroethylene) membrane | - |
| Glucose oxidase | Glucose | $SiO_2$ gel-carbon composite electrode on optical fibre | Amperometric |
| Glucose oxidase | Glucose | 3-aminopropyltriethoxy-silane, 2-(3,4-epoxycyclohexyl)ethyltrimethoxisilane | Amperometric |
| Glucose oxidase + ferrocene | Glucose | $SiO_2$ gel + polyelecrolyte | Amperometric |
| Horseradish peroxidase | $H_2O_2$ | Enzyme adsorption on gold nanoparticles chemiosorbed to a thiol-contg. sol-gel network | Amperometric |
| Horseradish peroxidase | $H_2O_2$ | Polypyrrole electrodeposited on sol-gel derived composite carbon electrode | Amperometric |
| Horseradish peroxidase + Hexacyanoferrate (II) | $H_2O_2$ | $SiO_2$ gel + natural polymer chitosan on C paste electrode | Amperometric |

Besides biosensors, Pierre presents some interesting applications of enzyme-based sol-gel biocatalysts in fine organic chemical synthesis. Table 4, based on 34 references from the period 1997-2003, includes some examples.[67]

*Table 4*

Some chemical synthesis reactions recently studied with sol-gel encapsulated enzymes

| Enzyme | Encapsulation medium | Reaction |
|---|---|---|
| Lipases from Burkholderia cepacia and Rhizomucor miehei | Phyllosilicate (clay) gel | Esterification with butanol, ethanol, isopropanol |
| Lipase from Burkholderia cepacia | Phyllosilicate (clay) gel | Esterification of restaurant grease |
| Lipases from Burkholderia cepacia and Rhizopus oryzae | Phyllosilicate (clay) gel | Esterification of glycerol with short, medium and long-chain fatty acids |
| Lipase from Burkholderia cepacia | Phyllosilicate (clay) gel | Conversion of grease or tallow to alkyl esters (biodiesel) |
| Lipase from Candida rugosa | Silica gel from PTMS and TMOS molar ratio = 4:1 | Esterification between ethanol and butyric acid in hexane |
| Lipase from Candida rugosa | Silica gel from PTMS and TMOS molar ratio = 4:1 + $Fe_3O_4$ | Esterification between ethanol and butyric acid in hexane |
| Various Lipases | Silica gel + $Fe_3O_4$ | Esterification of lauric acid by 1-octanol |
| Lipase from Candida rugosa | Silica gel in polyester fabric | Esterification of geraniol and acetic acid |
| Lipolase 100 L from Novo Nordisk | Silica gel | Esterification of stearic acid with aliphatic alcohols |
| Lipolase 100 L from Novo Nordisk | Adsorbed on silica particles from poly(methylhydroxysiloxane) + encapsulated in silica gel from PDMS | Esterification of stearic acid with aliphatic alcohols |
| Lipase from Rhizopus Javanicus | Silica gel with n-butyl functionalities, with enantiometric template on celite | Enantioselective esterification of glycidol with n-butyric acid |
| Lipolase 100L, Lipase PPL (Sigma), Lipase from Aspergilus niger | Silica gel and silicone polymeric gel | Esterification of stearic acid and corey lactone bisalc. |
| Lipase from Candida rugosa | Hybrid silicate gel | Esterification of menthol with butyric acid |
| Lipase from Mucor miehei + metal complex $RhCl[P(C_6H_5)_3]_3$ or $Rh_2Co_2(CO)$ | Separate silica gel domains | One pot esterification and hydrogenation of alcohols and acids |
| Lipase from Burkholderia cepacia | Silica gel with methyl, Z or isobutyl functionalities | Hydrolysis of soybean oil |
| Invertase | Mixed cellulose acetate and zirconium tetra-n-butoxide gel fibre | Hydrolysis of sucrose |

*Table 4* (continues)

*Table 4* (continued)

| | | |
|---|---|---|
| Various Lipases | Silica gel | Hydrolysis of esters |
| Uridine phosphorylase + purine nucleoside phosphorylase | Immobilization on hydrophobic epoxy resin hydrophilized by reaction with amino acids and on agarose gel | Transglycosylation in aq. soln. between a nucleoside and a purine or pyrimidine base |
| Alkaline and neutral proteases from Bacillus licheniformis | Silica gel from TEOS or sodium silicate | Transformation of wool |
| Alcohol dehydrogenase | Silica gel | Conversion of formaldehyde to methanol |
| Formate dehydrogenase + formaldehyde dehydrogenase + alcohol dehydrogenase | Silica gel | Conversion of carbon dioxide to methanol |
| β-glucuronidase and arylsulfatase | Silica gel particles, packing in a column | Deconjugation of urinary conjugates |
| Lipoxygenase | Phyllosilicate clays cross-linked with silica gel | Production of hydroperoxy derivatives of polyunsaturated fatty acids |
| Lipoxygenase | Silica gel + alginate | Oxidation of polyunsaturated fatty acids |
| Peroxidase | - | Crosslinking of poly(aspartic acid) hydrogels |
| Almond oxynitrilase | Silica gel + 3-gluconamidopropyl siloxane + methyl silicate | Trans-hydrocyanation of (2E,4E)-hexa-2,4-dienal to (1R,2E,4E)-1cyanohexa-2,4-dienal-1-ol |
| Lipase from Candida rugosa | Silica gel | Aminolysis of ethyl-2-chloropropianae |
| Lipase | Silica + $Fe_3O_4$ gel | Reaction of racemic 2-pentylamine with ethyl acetate |

Tables 3 and 4 represent only two examples confirming the validity of the affirmation that the list of biomaterials is in a continuous increasing. The review of Avnir and co-workers[69] which appeared this year, entitled "Recent bio-applications of sol-gel materials" is devoted to the most recent developments (2000-2005) of sol-gel materials at the interface with biology. It refers to the sol-gel immobilization of numerous proteins, enzymes and immune molecules and it is based on 148 references.

## SOME PERSONAL RESULTS REGARDING THE PREPARATION OF BIOMATERIALS BY THE SOL-GEL METHOD

### 1. A few specifications

Personal researches[48, 49, 55, 56, 59-61, 76, 77] in the domain of bio-composites obtained by the entrapment of enzymes in $SiO_2$ gels have had in view among other things, the comparative study of some biomaterials obtained by the both routes of the sol-gel method: the alkoxide and the aqueous (colloidal) one. These studies have demonstrated that in the case in which bio-molecules are entrapped, the resulted alcohol from the synthesis accomplished by the alkoxide route of the sol-gel method represents an undesirable product which can affect the enzyme (by distorting it), fact confirmed by the literature data.[53, 63, 64, 71, 78] The aqueous medium provided by the colloidal route of the sol-gel procedure not only ensures the activity of the enzymes but it can also improve their stability. This is the reason for which the presented studies have granted a special importance to the aqueous (colloidal) route of the sol-gel method.

The researches referring to the immobilization of different types of enzymes (glucose oxidase, respectively proteases) have been based on a previous experience in the sol-gel domain which has presumed a systematic study regarding the preparation of the amorphous silica matrices.[60, 79-84]

### 2. Studies regarding the preparation of amorphous silica sol-gel matrices for enzymes encapsulation

The gelation of the most common silica precursors in the colloidal (aqueous) route of the sol-gel method (respectively the colloidal silica and the sodium silicate solution) has been studied.[85]

In order to establish the influence of alkaline ions ($Na^+$) on the gel formation, four well known characterization methods have been used: pH-metry, viscosimetry, the complex formation method of the silicate ions with ammonium molybdate, and the IR spectroscopy.

Table 5 presents the systematized results of the comparative gelation processes performed on the two mentioned silica-based solutions both diluted at the same $SiO_2$ concentration (1 wt. %). Their gelation was performed at pH = 5 by destabilization with $HNO_3$ 25 wt. %. The selection of the $SiO_2$ concentration and of the pH value was imposed by the necessity to compare the gelation processes in reasonable limits of time.

*Table 5*

Comparative study of the gelation of some aqueous (colloidal) silicatic solutions having a 1% $SiO_2$ concentration, at pH =5

| The monitored parameter | The pursuit of the gelation process of a silicatic solution containing 1% $SiO_2$ at pH = 5 | |
|---|---|---|
| | Colloidal silica (CS) | Sodium silicate (SS) |
| pH | 5-6.94 | 5-5.51 |
| Viscosity | Newtonian fluid | Newtonian fluid in the first 5 hours from the start of the gelation process. As it gets closer to the gelation point, it becomes non-Newtonian. |
| Polymerization degree (PD) | (Initial PD > 4)<br>The dilution produces an advanced depolymerization, practically to the monomer (which is detectable even after 11 weeks from the start of the gelation process). The gelation takes place by the aggregation of the molecular species present in the system (it results the molecular stability of the colloidal silica sol). | (Initial PD = 6)<br>The dilution does not produce the depolymerization, the cyclohexasilicate representing the prevalent specie before as well as after the start of the gelation process. This structure is kepted near to the gelation point (~ 5 hours), when PD increases from 6 to 8 (dicyclotetrasilicate).<br>It could be concluded that the presence of the alkaline ions ($Na^+$) is responsible for the increase of the PD which corresponds also with the modification of the rheology of the system. |
| IR spectroscopy | For both silica-based solutions the aspect of the IR spectra changes from the viewpoint of the number and of the positions of the absorption bands as the gelation process proceeds. The IR spectra could be divided into two main regions: one between 4000 and 1500 $cm^{-1}$ corresponding to the absorption bands of water and $OH^-$ groups and the other under 1500 $cm^{-1}$ where the bands due to the various vibrations of Si-O-Si bonds could be founded. The gelation process can be monitored by following the increase of the intensity of the bands centred at 1100, 800, and 460 $cm^{-1}$ during the gelation time. These bands are ascribed to the asymmetric (1100 $cm^{-1}$) and symmetric (800 $cm^{-1}$) vibrations of the Si-O-Si bonds and to the rocking vibration (460 $cm^{-1}$) of the O-Si-O bond. The increase of their intensity strongly suggests the formation in time of the tri-dimensional network of the silica gel. | |
| | The absorption bands characteristic to the vibrations of Si-O-Si bonds could be observed only after a substantial time after the beginning of the gelation process (few weeks), proving that it proceeds with a very low rate. | The absorption bands characteristic to the vibrations of Si-O-Si bonds could be observed even from the beginning of the gelation process. This fact is in good agreement with the literature data which attribute to the alkaline ions the increase of the polymerization degree of the silicate groups.[86] This observation is sustained by the PD determinations. |

All the methods used in our study put in evidence that the presence of alkaline ions ($Na^+$) in the system essentially changes its behaviour. It was established that for the same $SiO_2$ concentration of the studied silica-based solution, the gelation rate is over 200 times higher in the case when $Na^+$ ions are present than in the case of their absence. The gelation time reduces from weeks to hours, which is explained by the significant decrease of the electrostatic repulsion between silicate groups that leads to their condensation.

Taking into account the conclusions of this study it becomes well to understand the reason for which all the sol-gel prepared biomaterials via the colloidal (aqueous) route presented further on have been obtained using a sodium silicate solution as silica precursor.

## 3. Glucose oxidase-based nanobiomaterials

Glucose oxidase (GOD) represents one of the most studied entrapped enzymes[48, 56, 61, 87-90] because of its importance in the medical domain. The monomeric molecule is a compact spheroid with approximate dimensions 6.0 x 5.2 x 3.7 nm.[91] The corresponding dimensions of the dimer are 7.0 x 5.5 x 8.0 nm.[91] In order to develop an analytical device based on visual detection, e.g. test strips for the glucose concentrations in blood or urine, the main enzymatic reaction, respectively the GOD reaction (which catalyzes the oxidation of D-glucose) has to be correlated with a second enzymatic reaction (catalyzed by peroxidase in the presence of different types and concentrations of chromogenic agents).

Despite the considerable number of attempts for GOD immobilization by the sol-gel method, various aspects of the process still deserve the interest of the researchers. One of them is the stability of the final product which represents the critical factor of the commercially availability either for a glucose sensor or for an analytical device, in particular the test-strips for diabetics.

Our researches[48, 56, 61] have been focused on studying the stability characteristics of the glucose oxidase entrapped by the sol-gel method in two different matrices issued either from tetraethylorthosilicate (TEOS) or from sodium silicate solution, in order to develop analytical devices based on immobilized GOD. Generally, the glucose sensor was reported to be obtained in two-step procedures, which involve in the first stage the simultaneous immobilization of the enzymes in a silica matrix, followed by the second stage, when the resulting transparent gel is impregnated with a chromogenic mixture (consisting either from 3-dimethylaminobenzoic acid and 3-methylbenzothiazolidone hydrazone hydrochloride[92, 93] or from 4-aminoantipyrine and p-hydroxybenzene sulphonate[32, 71, 78]).

In our study we have successfully experimented for the first time the preparation of both types of biomaterials (respectively those obtained with the silica gel matrix issued from TEOS and those issued from Na silicate solution) in one single step. So, the gelation of the silica sources was realized both in the presence of the enzymes (glucose oxidase and peroxidase) and of the chromogenic mixture (consisting in our case in 4-aminoantipyrine and p-hydroxybenzene sulphonate).

Our proposed one-step procedure for the sol-gel preparation of the GOD-based biomaterials has not only the advantage of simplifying the experiments by eliminating the second step in the device preparation, but also of improving the homogeneity of the analytical device by an uniform dispersion of all the enzymatic compounds into the whole matrix. Through this procedure the quantity of chromogenic reactants can be strictly controlled, which could be considered a contribution for the improvement of the reproducibility and repeatability of the final device.

The first experimental runs were directed to determine the adequate conditions for an optimal GOD immobilization from pH point of view in the *p*H range of 5.0-7.0, which must be correlated with the *p*H domain of the enzyme activity.[48] It was established that the activity of the entrapped GOD is optimal in the *p*H range of 6.0-7.0 for both types of matrices. The significant difference between the two sol-gel methods consists in the gelation times of the doped matrices. Thus, the inorganic ones need a gelation time from 2.5 to 360 minutes (depending on the *p*H value), while the organic require some days (1-4 days) for reaching the same stage of gelation.

The *p*H value of 6.5 was retained for further studies,[56, 61] being confirmed by the literature as corresponding to the maximum substrate response.[90]

Being well known the importance of the water for the bio-catalytic capacity and the general correlation between residual water and the enzymatic activity, a study of the drying process was accomplished, in order to improve the stability of immobilized GOD.[48] The controlled parameters of the drying process were the temperature, the humidity, and the thickness of the biomaterial layer. So, it was analyzed the activity of biomaterials obtained by both routes of the sol-gel process, which have been dried in different ways: at room temperature, either in 3 cm or in 1.5 cm thickness, in wet room, and in refrigerator. The different provenience of the sol-gel matrix has leaded to different results: for the biomaterials obtained through the alkoxide route of the sol-gel method, the best results corresponded to a drying process in wet room, while for the other ones (obtained via the colloidal sol-gel method) the drying at room temperature in 3 cm thickness layer is preferable.

Because the GOD concentration is responsible for the performance of the analytical device, both the activity and the stability of the two types of biomaterials have been studied[61] in a large range of GOD concentrations: 5-200 mg. In the 5-50 mg GOD domain, no matter the silica matrix nature, the increase of the enzyme concentration in the doped matrix was accompanied by an increased enzymatic activity of the biomaterial. A GOD concentration over 50 mg was not followed by a proportional increase of the enzymatic activity of the immobilized enzyme. Instead, the time-stability of the final product was significantly enhanced at the highest enzyme concentrations, which normally correspond to a high protein concentration. Table 6 presents the obtained results.

As it can be seen from Table 6, the entrapment of GOD in silica gel matrices derived from TEOS and from sodium silicate solution presents some differences depending on the nature of the matrix. Thus, the enzymatic activity and the time-stability of the doped materials are strongly influenced.

*Table 6*

The influence of GOD concentration on the stability of the two types of prepared biomaterials expressed as % of remanent enzymatic activity during the time

| Biomaterial* | Enzymatic activity [e.u.] | | | |
|---|---|---|---|---|
| | Initially | After 1 week | After 3 weeks | After 4.5 weeks |
| $O_{50}$ | 100 | 96.21 | 87.05 | 46.38 |
| $O_{100}$ | 100 | 59.88 | 54.12 | 56.64 |
| $O_{150}$ | 100 | 49.35 | 41.92 | 36.68 |
| $O_{200}$ | 100 | 72.96 | 69.33 | 66.01 |
| $I_{50}$ | 100 | 79.05 | - | 62.13 |
| $I_{100}$ | 100 | 100 | - | 80.40 |
| $I_{150}$ | 100 | 100 | 89.83 | 57.56 |
| $I_{200}$ | 100 | - | - | 90.59 |

* "O" suggests a silica matrix proceeded from an organic precursor (TEOS);
"I" suggests a silica matrix proceeded from an inorganic precursor (sodium silicate solution);
The subscript represents the GOD concentration of the sample

Although all the prepared GOD-based biomaterials correspond for future applications, e.g. test-strips for the glucose concentration determinations in blood or urine, we conclude that those obtained by the aqueous route of the sol-gel method seems to be better than the others (prepared via the alkoxide route).

## 4. Proteases-based nanobiomaterials

One of the most important domains of the industry is represented by the textile industry. In our days it is well known the fact that it represents one of the greatest pollutants, from both quantitative and qualitative point of views. To replace the present, classical technologies with some new, non-pollutant, "clean" ones which are not aggressive neither for the textile fibres nor for the ambient medium represents one of the most important targets for the scientific research.

The proteolithic enzymes, e.g. protease and proteinases are enzymes with overall dimensions between 23 and 25 nm, which under appropriate conditions specifically hydrolyze peptidic bonds from proteins. Being a natural complex of fibres, mainly composed from proteins (97 %) and lipids (1 %), the wool represents the ideal substrate for this class of enzymes.

Some personal studies (of preparation and characterization) regarding the use of proteases-based biomaterials in the wool industry (for washing and different treatments as bleaching and dying) will be presented.[49, 55, 59, 60, 76, 77] First of all a comparative study regarding the preparation of proteases-based sol-gel bio-materials by the two routes of the sol-gel process[60] was accomplished. The entrapped enzymes consisted from a mixture of some alkaline and neutral proteases biosynthesised from Bacillus licheniformis. The silica precursors were tetraethylorthosilicate (TEOS) in the alkoxide route of the sol-gel method, respectively a sodium silicate solution in the aqueous one. Table 7 presents the experimental conditions for preparing the mentioned bio-materials, together with some of their properties.

*Table 7*

Experimental conditions for preparing $SiO_2$ gels containing the proteasic mixture (ENZ) and some of their properties

| Bio-material* | Composition, [mL] | | | | Gelation conditions | | | Properties | |
|---|---|---|---|---|---|---|---|---|---|
| | $SiO_2$ | EtOH | $H_2O$ | ENZ | pH | T, [°C] | t, [h] | Struct. | Sp. surface aria, [$m^2/g$] |
| $O_1$ | 2.8 | 5 | 1 | 2 | 8.5 | 27 | 72 | amorph. | 97 |
| $O_2$ | 2.8 | 5 | 1 | 4.8 | 8.5 | 27 | 72 | amorph. | 131 |
| $I_1$ | 15 | - | - | 2.4 | 8.5 | 27 | inst. | amorph. | 203 |
| $I_2$ | 15 | - | - | 4.8 | 8.5 | 27 | inst. | amorph. | 142 |

* "O" suggests a silica matrix proceeded from an organic precursor (TEOS);
"I" suggests a silica matrix proceeded from an inorganic precursor (sodium silicate solution containing 3% $SiO_2$).

It is important to note that by the immobilization of the proteasic mixture in the silica gels a decrease of the optimum pH value range for activating the mixture of proteases from 9.5-11 to 8.5, the maintenance of

the enzymatic activity and a spectacular increase in the time stability of the enzymes (from 48 hours to > 5 months) were noticed. In fact, the possibility of the change of the optimum pH of an enzyme in the case of its immobilization is confirmed by the literature data[31] depending on the enzyme protein molecule and /or of the support.

SEM studies have demonstrated[60] the major difference existing between the two types of silica matrices obtained from TEOS or from sodium silicate even in the absence of the proteasic mixture. A totally different morphology of the silica gel obtained from various silica sources (TEOS or sodium silicate) can be observed. The observation remains valid also for the proteasic mixture containing silica gels issued from TEOS, respectively from sodium silicate.

The change of the morphology of the silica gel in the presence of the enzyme is obvious. More than that, the quantity of the proteasic mixture strongly influences the structure. It can be observed that samples containing a double concentration of enzyme present a sort of organization of the network (especially sample $I_2$). The SEM determinations are in good agreement with the specific surface area values presented in Table 7. The lower value of the specific surface area for the inorganic matrix at a higher content of enzyme is most probably due to the molecular association.

In order to complete the physico-chemical characterization of the prepared biomaterials and to prove the entrapment of the enzymes in the silica matrices, the IR characterization of the proteasic mixture and of the four samples has been accomplished.[60] The spectrum of the proteasic mixture (ENZ) presents a large, characteristic absorption band in the interval 3600-3050 $cm^{-1}$ due to OH and NH stretching. The 2950 $cm^{-1}$ shoulder for the asymmetrical C-H stretching and the 2100 $cm^{-1}$ overtone region, obtained from the combination of the asymmetrical $NH_3^+$ bending and $NH_3^+$ torsional oscillations are also present. Other characteristic absorption bands of the enzymes are observed at the following values ($cm^{-1}$): 1640m ($\nu_{C-O}$), and 1280s ($\nu_{C-O}$). In the range where enzymes and silica matrix IR bands overlap the characteristic absorption of the $SiO_2$ gel at 1100 and 800 $cm^{-1}$ (corresponding to $\nu_{Si-O-Si}$ asymmetric, respectively symmetric bond stretching vibration) and at 960 $cm^{-1}$ (attributed to $\nu_{Si-O-H}$ stretching vibration) are evident.

All the immobilized enzymes samples present a large absorption band in the range 3600-3050 $cm^{-1}$ due to OH (matrix + enzyme + $H_2O$) and NH (enzyme) stretching, putting in evidence the intermolecular hydrogen bonding. Other characteristic absorption of the enzyme observed in the immobilized samples are the shoulders at 2950 $cm^{-1}$ (especially visible in the $O_1$ and $O_2$ spectra, being attributed to the aliphatic C-H bond from the $C_2H_5$ groups proceeded from the ethanol used as solvent in the alkoxide route of the sol-gel process) and the following values ($cm^{-1}$): 1640m ($\nu_{C-O}$), and 1280s ($\nu_{C-O}$). In the $O_1$ and $O_2$ spectra, because of the organic origin of the silica matrix, a strong absorption in the region 1200-1100 $cm^{-1}$ can be noticed, which is due to the C-O stretching band characteristic for the ether functional groups ($C_2H_5$-O) of the matrix. It overlaps the 1100 band assigned to the Si-O-Si vibration.

In conclusion the IR spectra agree with an immobilized enzyme structure showing the characteristic absorption bands for the proteolytic enzymes together with those of the silica polymer matrix.

Both types of presented biomaterials have been tested in the textile biotechnology field in a series of experiments in order to establish the effects of the immobilized enzymatic mixture in wool industry.[49, 59, 76, 77] In order to establish the catalytic activity of the four synthesized biomaterials on the wool, wool yarn samples with linear density of 113 tex, prepared from 25 μm fibres have been used.[59] The wool samples have been treated with mixtures of immobilized enzymes (catalyst) in phosphate buffered solutions at different pH values (6.0, 7.5, and 8.5). Blank samples containing only wool and phosphate solution were also prepared for comparison. The effect of the enzymes on the wool fibres was a measurable one and it was expressed by the weight loss of the wool samples, which were determined in standard conditions using a semi-micro-analytical balance MB-C-01 type. Figure 1 presents the obtained weight losses in phosphate buffered solutions of the sol-gel prepared biomaterials ($I_1$, $I_2$, $O_1$, and $O_2$), depending on the pH value.

From the obtained results it can be concluded that for all the prepared biomaterials the best experimental results were obtained for pH = 7.5, at which the weight losses values are enough to modify the characteristics of the fibre in economically advantageous conditions (not so high to damage the fibre, as at pH = 6.0 or not so small to let it unchanged, as at pH = 8.5). From Figure 1 it can be observed that for the biomaterials prepared by the alkoxide route of the sol-gel method ($O_1$ and $O_2$) the increase of the enzyme content led to a higher activity (for all pH values), while in the case of $I_1$ and $I_2$ (prepared by the aqueous route of the sol-gel method), the effect was opposite. These results are in good agreement with the specific

surface area measurements and scanning electron microscopy determinations presented above. Reverting to the value of pH which was found to be the most advantageous for the treatment of the wool fibres, Figure 2 presents a significant example of effect of immobilized enzymes action on wool in the treatment at pH = 7.5. It refers to the biomaterials named $I_2$ proceeded by the colloidal (aqueous) route of the sol-gel method.

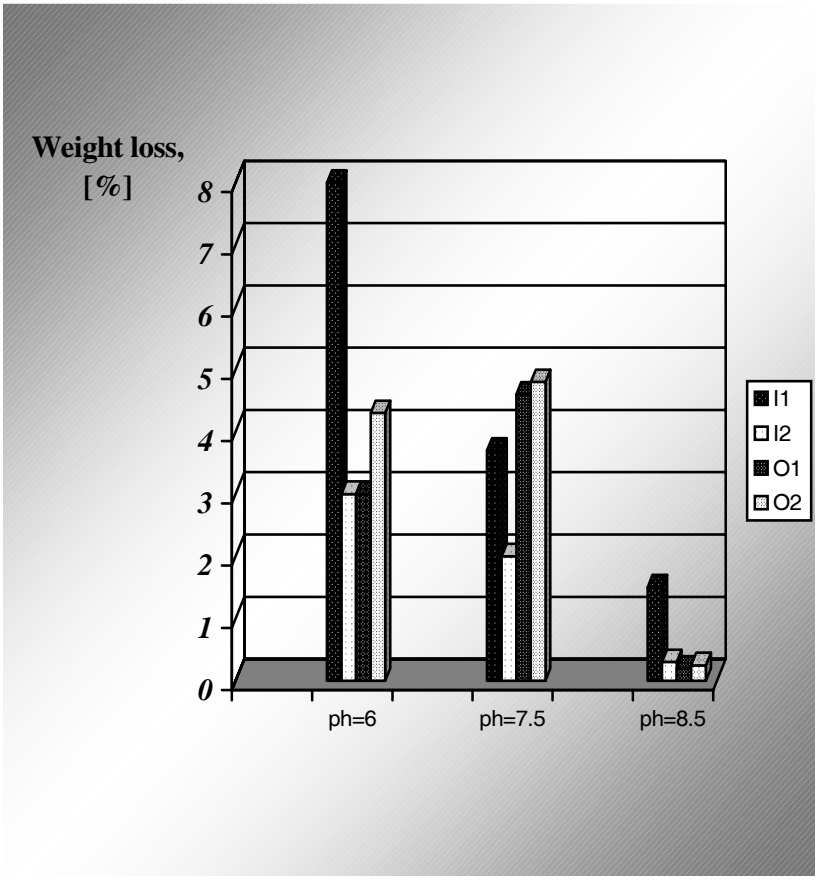


Fig. 1 – Wool weight losses in phosphate buffered solutions of the prepared biomaterials at different pH values.

Based on the obtained results it was tested a combined treatment on wool with $H_2O_2$ and immobilized enzymes in order to establish the influence of biocatalysts in wool bleaching. The effect of the simultaneously action of both chemical and biochemical agents on wool fibre was also estimated by determining the weight losses.

The experimental results have evidenced the fact that the nature of the matrix influences the properties of the prepared biomaterials. Even if all tests proved that the enzymatic treatments of wool fibres led to a better handle and to a higher degree of whiteness compared with the same samples in the absence of the treatment, the best results have been obtained for those biomaterials which were prepared by the aqueous route of the sol-gel method ($I_1$ and $I_2$), compared with $O_1$ and $O_2$ (prepared by the alkoxide route). In fact, further similar studies have continued only on samples proceeded by the aqueous route of the sol-gel method, respectively samples for which the silica precursor was a sodium silicate solution.[55] Paper[76] presents the obtained results regarding the improvement of the wool quality as a consequence of an enzymatic treatment with immobilized enzymes. It evidences the fact that using the right conditions (type of catalyst and pH) the control of the weight loss of the wool fibre can be obtained. It refers also to both types of biomaterials (with the same different silica precursors, depending of the chosen route of the sol-gel method: TEOS and sodium silicate) but the tested enzymes were Trypsin from Merck and a commercial alkaline protease from the Serine family from Aachen. The wool sample treated with the mentioned enzymes improved substantially their whiteness and handle properties. In order to establish the improvement of the dyeing properties of the wool fibres as a result of an enzymatic pre-treatment and based on the established conclusions of the previous works, the fixation of an anthraquinone dye Bemacid Brilliant Blue 2R 200% on wool samples

treated with immobilized enzymes was studied.[49, 77] Table 8 presents the weight losses and the bath exhaustions when pre-treated enzymatic wool fibres were treated with Bemacid Brilliant Blue 2R dye.

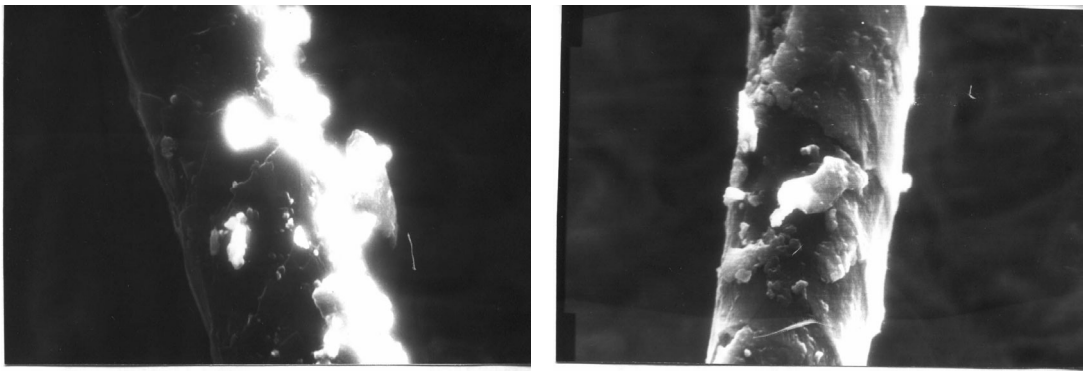

Fig. 2 – SEM images (x 1.800) of the wool fibre before (a) and after thermal treatment with biocatalyst $I_2$ at pH=7.5 (b).

*Table 8*

The weight losses and the bath exhaustions after enzymatic treatment of wool fibres treated with Bemacid Brilliant Blue 2R dye

| pH of the treatment | pH = 6 | | pH = 7.5 | | pH = 8.5 | |
|---|---|---|---|---|---|---|
| Sample | ΔW*, [%] | BE•, [%] | ΔW, [%] | BE, [%] | ΔW, [%] | BE, [%] |
| $O_1$ | 3.91 | 73.50 | 2.45 | 65.30 | 4.48 | 72.00 |
| $O_2$ | 4.17 | 76.00 | 3.30 | 73.40 | 5.28 | 73.60 |
| $I_1$ | 3.33 | 76.80 | 2.54 | 72.80 | 5.04 | 77.80 |
| $I_2$ | 4.13 | 79.30 | 1.40 | 71.60 | 3.61 | 73.30 |

* ΔW - the weight loss, according to the known formula: $\Delta W = 100(W_i - W_f)/W_i$
where: $W_i$ = the initial weight;
$W_f$ = the final weight.
• BE - the bath exhaustion, according to the formula: $BE = 100(Abs_i - Abs_f)/Abs_i$
where: $Abs_i$ = the initial absorbance;
$Abs_f$ = the final absorbance.

As it can be seen from Table 8, the experimental results prove again the superiority of the biomaterials obtained by the colloidal route of the sol-gel method, compared to those resulted by the alkoxide one.

## CONCLUSIONS

The present work refers to a sub-domain of the sol-gel prepared biomaterials, respectively that in which the biomolecule is represented by an enzyme. After a general presentation of what sol-gel process and biomaterial are, it focuses on some personal results of its author in the mentioned field. Concerning these results, there are some aspects to retain:

Bio-materials consisting of different enzymes entrapped in silica matrices have been prepared, using both routes of the sol-gel method (the alkoxide and the colloidal one).

All the prepared samples have been realised with personal compositions, none of them was taken over the literature.

The used enzymes consisted from: (1) glucose oxidase, resulting biomaterials with possible applications in the medical domain (for the determination of the glucose concentration from blood and/or urine), and (2) mixtures of proteases, resulting biomaterials with possible applications in the textile industry (for washing and different treatments as bleaching or dyeing of the wool).

The prepared samples have been characterized using the following methods: scanning electron microscopy (SEM), infrared (IR) and UV-Vis spectroscopy, the BET method for the specific surface area measurements, and the corresponding methods for the enzymatic activity determinations, depending on the used enzyme.

A general conclusion from all the presented studies consists in the recommendation to prepare biomaterials via the aqueous route of the sol-gel method, compared to the alkoxide one.

## REFERENCES


1. T. Lopez and E. Sanchez, *React. Kinetic. Catal. Lett.*, **1992**, *48*, 295.
2. T. Lopez, R. Gomez, O. Novaro, A. Ramirez Solis, E. Sanchez Mora, S. Castillo, E. Poulain and J. M. Martinez Magadan, *J. Catalysis*, **1993**, *141*, 114.
3. O. K. Filho and M. A. Aegerter, *J. Non-Cryst. Solids*, **1988**, *105*, 191.
4. A. C. Pierre, "Introduction au procédés sol-gel", Editions SEPTIMA, Paris, 1992.
5. H. Schmidt, *J. Non.-Cryst. Solids*, **1988**, *100*, 51.
6. T. Lopez, J. Mendez, T. Zamudio and M. Villa, *Mater. Chem. Phys*, **1990**, *30*, 161.
7. D. A. Ward and E. I. Ko, *Ind. Eng Chem. Res*, **1995**, *34*, 421.
8. R. K. Iler, "The Chemistry of Silica", Ed. John Wiley & Sons, 1979.
9. J. Zarzycki, *J. Non-Cryst. Solids*, **1990**, *121*, 110.
10. L. L. Hench and J. K. West, *Chem. Rev.*, **1990**, *90*, 33.
11. K. Almdal, J. Dyre, S. Hvdit and O. Kramer, *Polymer Gels and Networks*, **1993**, *1*, 1.
12. S. Sakka and K. Kamiya, *J. Non-Cryst. Solids*, **1982**, *48*, 31.
13. P. Yu, H. Liu and Y. Wang, *J. Non-Cryst. Solids*, **1982**, *52*, 511.
14. W. H. Dokter, T. P. M. Beelen, H. F. van Garderen and R. A. van Santen, *Mat. Res. Soc. Symp. Proc*., 1994, *346*, 217.
15. T. P. M. Beelen, W. H. Dokter, H. F. van Garderen, R. A. van Santen and E. Pantos, *Prep.of Catalysts*, **1995**, *VI*, 33.
16. C. J. Brinker, D. M. Smith, R. Deshpande, P. M. Davis, S. Hietala, G. C. Frye, C. S. Ashley and R. A. Assink, *Catal. Today*, **1992**, *14*, 155.
17. M. Zaharescu, In *Proceedings at The 21 Session of Scientific Communications*, Oct. 25-27, Călimănești - Căciulata, **1995**.
18. K. R. Andersson, L. S. Dent Glasser and D. M. Smith, *ACS Washington D.C., Symposium Series,* **1982**, 115-132.
19. K. Shimada and T. Tarutani, *Bull. Chem. Soc. Jpn.*, **1980**, *53*, 3488-3491.
20. T. P. M. Beelen, W. H. Dokter, H. F. van Garderen and R. A. van Santen, *Adv. in Colloid and Interface Sci.*, **1994**, *50*, 23.
21. D. Kinrade and T. W. Swaddle, *Inorg. Chem.*, **1988**, *27*, 4253-4259.
22. D. Kinrade and T. W. Swaddle, *Inorg. Chem.*, **1988**, *27*, 4259-4264.
23. R. K. Iler, *A.C.S. Washington D.C. Symposium Series*, **1982**, *194*, 95-114.
24. D. Kinrade and D. L. Pole, *Inorg. Chem.*, **1992**, *31*, 4558-4563.
25. J. K. West, B. F. Zhu, Y. C. Ceng and L. L. Hench, *J. Non-Cryst. Solids*, **1990**, *121*, 51.
26. J. D. Mackenzie, *J. Non-Cryst. Solids*, **1982**, *48*, 1.
27. C. J. Brinker and G. W. Scherer, "Sol-Gel Science: The Physics and Chemistry of Sol-Gel Processing", Academic Press, New-York, 1990.
28. S. Komarneni, *J. Mater. Chem.*, **1992**, *2*, 1219.
29. G. Ennas, A. Musinu, G. Piccaluga, D. Zedda, D. Gatteschi, C. Sangregorio, J. L. Stanger, G. Concas and G. Spano, *Chem. Mater.*, **1998**, *10*, 495.
30. D. Avnir, D. Levy and R. Reinsfeld, *J. Phys. Chem.*, **1984**, *88*, 5956.
31. "Biotechnology" (ed. by H.-J. Rehm and G. Reed), vol.7a, Vol. Editor J.F. Kennedy, **1987**, p. 347, 393, 394, 11-13, 395, 467, 506, 349.
32. J. I. Zink, S. A. Yamanaka, L. M. Ellerby, J. S. Valentine, F. Nishida and B. Dunn, *J. Sol-Gel Sci.Tech.*, **1994**, *2*, 791-795.
33. P. Audebert and C. Sanchez, *J. Sol-Gel Sci. Tech.*, **1994**, *2*, 809-812.
34. S. A. Yamanaka, N. P. Nguyen, L. M. Ellerby, B. Dunn, J. S. Valentine and J. I. Zink, *J. Sol-Gel Sci.Tech.*, **1994**, *2*, 827.
35. B. C. Dave, B. Dunn, J. S. Valentine and J. I. Zink, *ACS Symposium Series*, **1995**, *585*, 351.
36. M. T. Reetz, A. Zonta and J. Simpelkamp, *Angew. Chem. Int. Ed. Engl.*, **1995**, *34*, 301-303.
37. M. T. Reetz, A. Zonta and J. Simpelkamp, *Biotech. & Bioeng.*, **1996**, *49*, 527-534.
38. M. T. Reetz, *Adv. Mater.*, **1997**, *9*, 943-954.
39. J. Lin and C. W. Brown, *Trends in Anal. Chem.*, **1997**, *16*, 200-211.
40. G. Kuncová and M. Šivel, *J. Sol-Gel Sci. Tech.*, **1997**, *8*, 667-671.
41. F. Akbarian, A. Lin, B. S. Dunn, J. S. Valentine and J. I. Zink, *J. Sol-Gel Sci. Tech.*, **1997**, *8*, 1067.
42. C. Roux, J. Livage, K. Farhati and L. Monjour, *J. Sol-Gel Sci. Tech.*, **1997**, *8*, 663.
43. B. C. Dave, J. M. Miller, B. Dunn, J. S. Valentine and J. I. Zink, *J. Sol-Gel Sci. Tech.*, **1997**, *8*, 629-634.
44. B. Dunn, J. M. Miller, B. C. Dave, J. S. Valentine and J. I. Zink, *Acta Mater.*, **1998**, *46*, 737-741.
45. I. Gill and A. Ballesteros, *J. Am. Chem. Soc.*, **1998**, *120*, 8587-8598.
46. U. Georgi, H. Graebner, G. Roewer and G. Wolf, *J. Sol-Gel Sci. Tech.*, **1998**, *13*, 295-298.
47. D.M. Liu, I.W. Chen, *Acta Mater.*, **1999**, *47(18)*, 4535-4544.
48. M. Arsene, D. L. Bordeianu, L. Stanciu, C. Pârlog and M. Răileanu, In *Proceedings at The13th Conference on Glass and Ceramics*, Varna, Sept. 29 - Oct. 1, Vol.1: Glass, **1999**, 345-350.
49. M. D. Stănescu, M. S. Bucur, M. Răileanu and M. Badea, *Sci. and Tech. Bull., Serie: Textile Science,"Aurel Vlaicu" Univ. Arad,*, **2000**, *6*, 20-26.
50. S. Fennouh, S. Guyon, J. Livage and C. Roux, *J. Sol-Gel Sci. Tech.*, **2000**, *19*, 647.
51. T. Coradin, E. Mercey, L. Lisnard and J. Livage, *Chem. Commun.*, **2001**, 2496.
52. T. Coradin and J. Livage, *Colloids and Surfaces B: Biointerfaces*, **2001**, *21*, 329.
53. A. Coiffier, T. Coradin, C. Roux, O. M. M. Bouvet and J. Livage, *J. Mater. Chem.*, **2001**, *11*, 2039-2044.
54. J. Livage, T. Coradin and C. Roux, *J. Phys. Condens. Matter.*, **2001**, *13*, R673-R691.
55. M. S. Bucur, M. D. Stănescu and M. Răileanu, In *Proceedings at The International Meeting of Textile Chemists and Colourists*, June 12-13, Budapest, Hungary, **2001**, 48-53.
56. M. Răileanu, M. Arsene, L. Stanciu, L. Bordeianu and M. Zaharescu, In *Proceedings at The International Semiconductor Conference*, 24th edition, Oct. 9-13, Sinaia, Roumania, **2001**, 281-284.

57. N. Nassif, O. Bouvet, M. N. Rager, C. Roux, T. Coradin and J. Livage, *Nat. Mater.*, **2002**, *1*, 42-44.
58. T. Coradin, O. Durupthy and J. Livage, *Langmuir*, **2002**, *18*, 2331.
59. M. Răileanu, L.Stanciu, M.Badea, M. Stănescu and L.Bordeianu, *Univ. Politehnica Buc., Scientific Bulletin, Series B: Chem. and Mat.Sci.*, **2002**, *64*, 55-62.
60. M. Răileanu, L. Stanciu, C. Pârlog, L. Bordeianu, M. Stănescu and M. Badea, *Rev. Roum. Chim.*, **2002**, *47*, 533-538.
61. M.Arsene, L. Stanciu, C. Pârlog, L. Bordeianu and M. Răileanu, *Rev. Roum. Chim.*, **2002**, *47*, 1267-1271.
62. S. Furukawa, T. Ono, H. Ijima and K. Kawakami, *Biotechnol. Lett.*, **2002**, *24*, 13-16.
63. N. Nassif, C. Roux, T. Coradin, M. N. Rager, O. M. M. Bouvet and J. Livage, *J. Mater. Chem.*, **2003**, *13*, 203-208.
64. T. Coradin, A.Coupé and J. Livage, *Colloids & Surfaces B: Biointerfaces*, **2003**, *29*, 189-196.
65. T. Coradin and J. Livage, *C. R. Chimie*, **2003**, *6*, 147-152.
66. N. Nassif, A. Coiffier, T. Coradin, C. Roux and J. Livage, *J. Sol-Gel Sci. Tech.*, **2003**, *26*, 1141-1144.
67. A. C. Pierre, *Biocatalysis and Biotransformation*, **2004**, *22*, 145-170.
68. N. Nassif, C. Roux, T. Coradin, O. M. M. Bouvet and J. Livage, *J. Mater. Chem.*, **2004**, *14*, 2264.
69. D. Avnir, T. Coradin, O. Lev and J. Livage, *J. Mater. Chem.*, **2006**, *16*, 1013-1030.
70. A. M. Hartnett, C. M. Ingersoll, G. A. Baker and F. V. Bright, *Anal. Chem.*, **1999**, *71*, 1215.
71. B. C. Dave, B. Dunn, J. S. Valentine and J. I. Zink, *Anal. Chem.*, **1994**, *66*, 1120A-1127A.
72. D. Avnir, *Acc. Chem. Res.*, **1995**, *28*, 328-334.
73. O. S. Wolfbeis, I. Oehme, N. Pokovskaya and I. Klimant, *Biosensors &Bioelectronics*, **2000**, *15*, 69.
74. H. Böttcher, *J. Prakt. Chem.*, **2000**, *342*, 427.
75. D. Avnir, L. C. Klein, D. Levy, U. Schubert and A. B. Wojcik, "The chemistry of organic silicon compounds", Vol. 2, Edited by Z. Rappoport and Y. Apeloig, John Wiley & Sons Ltd, 1998.
76. M. D. Stănescu, S. Mihuţă, M. S. Bucur, M. Răileanu and D. Popovici, "Advances in Biotechnology for Textile Processing", Edited by I.R. Hardin, D.E. Akin, and S.J. Wilson, University of Georgia, 2002, p. 109-116.
77. M. D. Stănescu, M. S. Bucur, M. Pustianu, S. Mihuţă and M. Răileanu, *DWI REPORTS, Future concepts for production and function, Aachener Textiltagung, Textile Conference*, Nov. 27-28, 2002, 465-468.
78. J.I. Zink, J.S. Valentine, B. Dunn, *New J. Chem.*, **1994**, *18*, 1109-1115.
79. M. Zaharescu, E. Pop, M. Răileanu, S. Mihaiu, R. Ganea and L. Milea, In *Proceedings at The Second Technical- Scientific Symposium "Zeolites in the Modern Technology"*, Iaşi, 1987, 143-148.
80. M. Zaharescu, M. Răileanu, S. Mihaiu, M. Stan and L. Constantinescu, *Rev. Chimie (Bucharest)*, **1991**, *42*, 406-410.
81. M. Zaharescu, M. Răileanu, S. Mihaiu and A. Vasilescu, *Rev. Chimie (Bucharest)*, **1992**, *43*, 557-560.
82. M. Zaharescu, R. Russu, M. Răileanu, M. Mihaiu and A. Vişan, *Rev. Chimie (Bucharest)*, **1996**, *47*, 533-538.
83. M. Răileanu, M. Zaharescu, M. Mihaiu, A. Vişan and R. Russu, *Progress in Catal., J. Rom. Catal. Soc.,* **1996**, *5*, 77-84.
84. M. Răileanu, L. Stanciu, H. Paven and M. Zaharescu, In *Proceedings at The Second Balkan Conference of Glass Science and Technology, The 14th Conference of Glass and Ceramics*, Sept. 24-28, Varna, Bulgaria, 2002; Editors: B. Samuneva, S. Bachvarov, I. Gutzov, Y. Dimitriev, Publishing House Science Invest Sofia, vol. 1- Glass, 2004, 450-455.
85. M. Răileanu, L. Stanciu, G. Tănase, I. Trandafir and M. Zaharescu, *Rev. Roum. Chim.*, **2002**, *47*, 455-460.
86. F. Gaboriaud, D. Chaumont, A. Nonat, B. Hanquet and A. Craeivich, *J. Sol-Gel Sci. Tech.*, **1998**, *13*, 353-358.
87. M. E. Tess and A. J. Cox, *J. Pharm. Biomed. Anal.*, **1999**, *19*, 55-68.
88. P. C. Panday, S. Upadhyay and H. C. Pathak, *Sensors and Actuators*, **1999**, *B 60*, 83-89.
89. S. de Marcos, J. Galindo, J. F. Sierra, J. Galban and J. R. Castillo, *Sensors and Actuators*, **1999**, *B 57*, 227-232.
90. Z. Liu, B. Liu, M. Zhang, J. Kong and J. Deng, *Anal. Chim. Acta*, **1999**, *392*, 135-141.
91. H. Tsuge and H. Mitsuda, *J. Biochem. (Tokyo)*, **1973**, *73*, 199.
92. S. Braun, S. Shtelzer, S. Rappoport, D. Avnir and M. Ottolenghi, *J. Non-Cryst. Solids*, **1992**, *147&148*, 739-743.
93. D. Avnir, S. Braun, O. Lev and M. Ottolenghi, *SPIE Sol-Gel Optics*-II, **1992**, *1758*, 456-463.