

Sol-gel hybrid materials for bioencapsulation

G. Chernev¹, B. Samuneva¹, P. Djambaski¹, I.M.M. Salvado²,
L. Kabaivanova³, E. Emanuilova³

¹Department of Silicate Technology, University of Chemical
Technology and Metallurgy, Sofia, Bulgaria

²Department of Ceramic and Glass Technology, CICECO,
University of Aveiro, Portugal

³Institute of Microbiology, Sofia, Bulgarian Academy of Sciences

georgi_chernev@yahoo.com



Introduction

A new field in the sol-gel technology is the synthesis of nanocomposite hybrid materials simultaneously containing organic and inorganic components. Such kind of materials is a subject of intensive investigations because they represent significant interest for the structural chemistry for studying their physical and chemical properties and potential applications in electronics, optics, microbiology, medicine and pharmacology (Carturan G. *et al.*, 1998, Kang E. *et al.*, 2004, Ogoshi T., *et al.*, 2005) Inorganic-organic hybrid materials are increasingly important due to their extraordinary properties, which arise from the synergism between the properties of the components (Hamano Y. *et al.*, 2004, Schubert U *et al.*, 2003).

The most popular precursors used for the synthesis of silicate hybrid materials assuring SiO₂ introduction are: TEOS, TMOS, MTES and others (Shchipunov A. *et al.*, 2005).

One of the most interesting and important problems in the study of hybrid nanocomposite materials is their structure analysis. Following the correlation between its evolution and the composition-structure-property dependence, as well as the processes of aggregation and development of self-organized or self-assembled structures. Up to now there is not enough information about these structures and the processes occurring during their formation are very important (Chen J. *et al.*, 2004, Sgarbi N. *et al.*, 2004, Tan X. *et al.*, 2005, Shchipunov A. *et al.*, 2005).

For the synthesis of hybrid materials the choice of the type and quantity of precursors and the organic components is also of importance, as well as the conditions for their synthesis (Valle K. *et al.*, 2006).

The hybrid nanomaterials can be successfully applied as carriers for immobilization of different biomolecules, including whole bacterial cells. Thus created biocatalysts could be applied for different purposes, starting from ethanol production up to processes of bioremediation-treating of contaminated sites (waters and soils) polluted as a result from different industrial processes by microorganisms, mainly bacteria (Chen T. *et al.*, 2003, Gill *et al.*, 2000)

Materials and Methods

In our previous works we have compared pure silica matrices with inorganic-organic hybrid matrices obtained by using different inorganic precursors (TEOS, TMOS, ETMS, MTES) and an organic compound such as: gelatin, PAAG, agar (Chernev G. *et al.*, 2005, Chernev G. *et al.*, 2006).

In the present work the research results on sol-gel synthesis, structure and application of hybrid nanocomposites containing different quantity of carrageenan (5, 10, 15 and 20 mol %) are described and discussed. The samples have been prepared at room temperature as films. Silicon precursors tetraethylortosilicate (TEOS) and methyltriethoxysilane (MTES) purchased by "ABCR" have been used. A poly-step sol-gel procedure is used at strictly controlled conditions in order to obtain the desired nanostructured materials.

For studying the structure of synthesized hybrids the following techniques have been used: FT-IR (IR- MATSON 7000-FTIR spectrometer), XRD (X-ray PW1730/10 diffractometer), BET-Analysis

(Gemini 2370 V5.00), EDS (RONTEC EDS System), SEM (Philips-515), AFM (NanoScope IIIA Tapping Mode™) and Roughness analysis.

The bacterial strain used in the present study was deposited in the NBIMCC-Bulgaria №8021/2001 (Emanuilova *et al.*, 2002). *Bacillus sp.* UG-5B with 4-cyanopyridine degrading activity was cultivated in a medium, containing 20 mM 4-cyanopyridine, pH 7.5 at 50°C.

Results and Discussion

The results from the XRD-analysis show that all the studied hybrids are in amorphous state (Fig. 1).

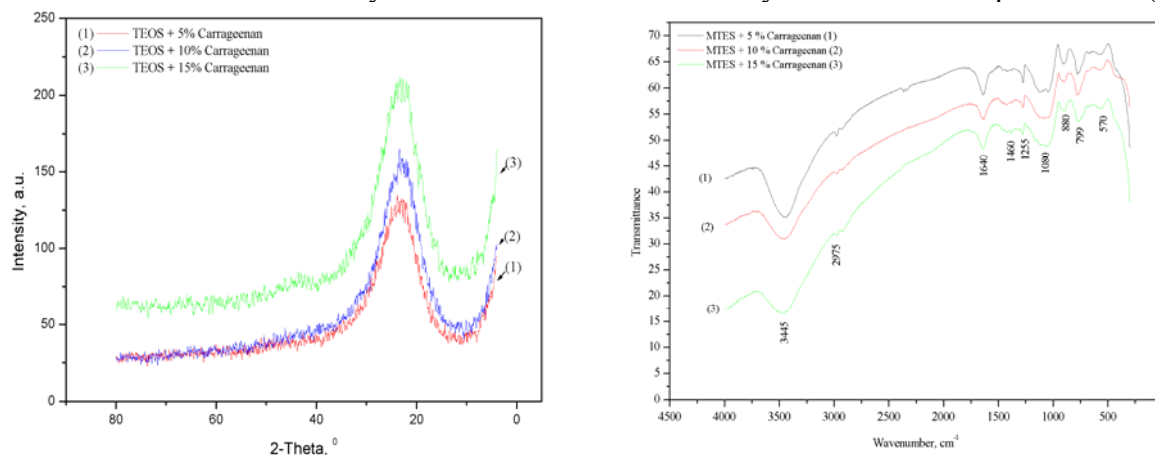


Fig.1 XRD patterns of hybrid nanomaterials Fig.2 FT-IR spectra of hybrid nanomaterials

The FT-IR spectra of synthesized inorganic-organic materials are shown that in all samples bands at 1080 cm^{-1} , 790 cm^{-1} and 480 cm^{-1} are observed. They are assigned to ν_{as} , ν_{s} and δ of Si-O-Si vibrations, but at the same time the band at 1080 cm^{-1} can be related to the presence of Si-O-C, C-O-C and Si-C bonds. The band at 960 cm^{-1} is due to a stretching Si-OH vibration. The band at 1439 cm^{-1} is assigned to C-O-H vibrations. The characteristic bands at around 3450 cm^{-1} and at 1620 cm^{-1} assigned to H-O-H vibration can also be detected. In all samples synthesized with MTES the absorption band at 2975 cm^{-1} , 1255 cm^{-1} , 880 cm^{-1} and 690 cm^{-1} , due to the presence of Si-O-R (CH_3 and C_2H_5) and Si-C bonds have been observed by FT-IR spectra. This fact directly proves the presence of strong chemical bonds between inorganic and organic parts of these hybrid materials (Fig. 2).

The surface structure of the hybrids has been observed by SEM. It can be seen that the surface appears to be smooth and there are no microcavities in the samples. The presence of a hybrid nanostructure with well-defined nanounits and their aggregates, formed by self-organizing processes, is clearly observed by AFM studies. The size of nanoparticles is from 3 to 9 nm (Fig. 3a and 4a). Figures 3b and 4b show the height distribution profiles of surfaces roughness. The histograms of the surface height distribution profiles, obtained from AFM images, show that all of the organic-inorganic polymer hybrid samples have surfaces with irregularities of quite small height. In the cases of TEOS and 5% carrageenan and TEOS and 20% carrageenan, the largest observed heights were 10,9 nm and 7,6 nm, respectively.

From the data of BET analysis it has been established that the surface area is in the range of 90 to $310\text{ m}^2/\text{g}$. The results clearly show that the surface area decreases with the increase of the percent of organic part. The surface morphology and structure of nanobuilding blocks in each synthesized hybrid is different and depends on its chemical composition. In the sample the nanoparticles are well distributed in the entire hybrid matrix with a lower degree of aggregation, while the sample prepared with MTES showed that the nanoparticles are self-organized and distributed as clusters in the matrix. Although all being amorphous, quite different self-organized structures can be observed in these hybrids.

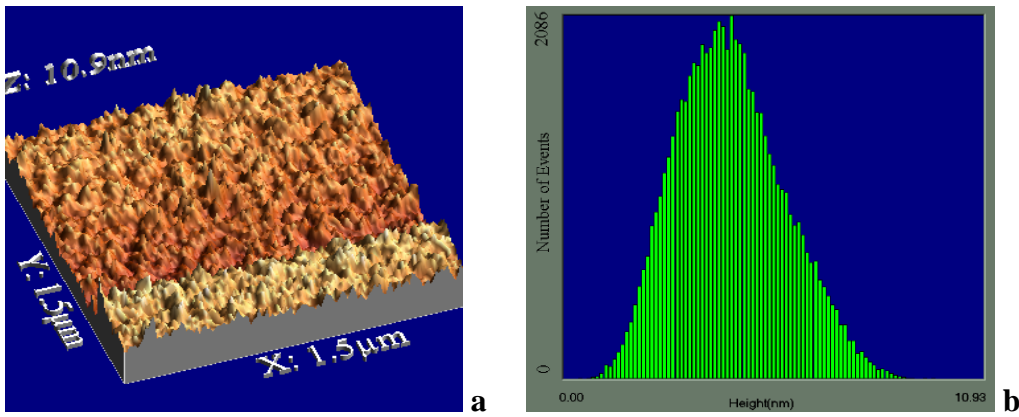


Fig. 3. AFM image (a), height distribution profile of surface roughness (b) of hybrid nanomaterials containing TEOS and 5% carrageenan,

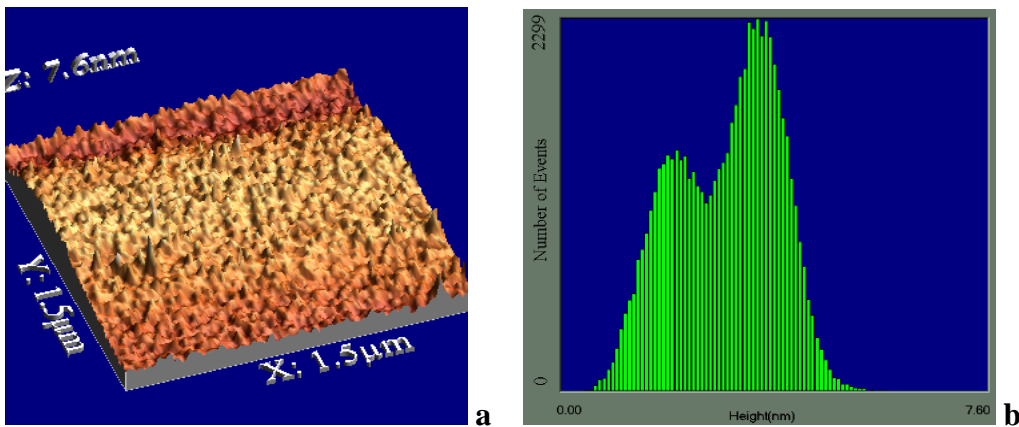


Fig. 4. AFM image (a), height distribution profile of surface roughness (b) of hybrid nanomaterials containing TEOS and 5% carrageenan,

A column bioreactor was filled with 45g of hybrid materials with entrapped bacterial cells. The process is carried out at 55⁰C for the period of 3 hours. The remaining not degraded quantity of the substrate was treated again for another 3 hours to achieve better degradation.

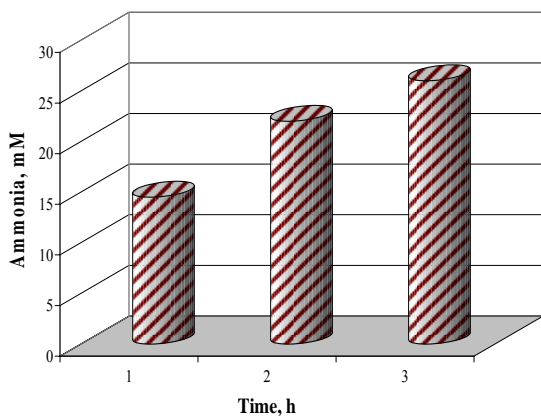


Fig. Degradation of fumaronitrile for the period of three hours-first step

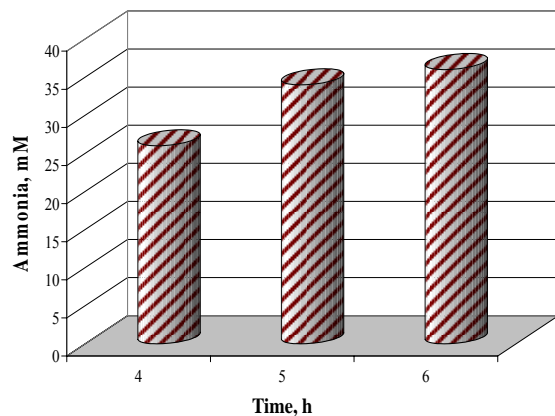


Fig. Degradation of fumaronitrile for the period of three hours-second step

The immobilized cells from the investigated strain are able to degrade structurally different nitrile compounds, such as benzonitrile and 4-cyanopyridine. In the present work we report on the biodegradation of fumaronitrile – toxic cyanide compound, possessing two cyano-groups. It's

degradation was achieved by a two-step process in a column bioreactor, using biocatalysts, obtained by encapsulation of the microbial cells in the system TEOS-carrageenan. The process was carried out at 55°C and biodegradation of 26 mM fumaronitrile at the first step for 3 hours, and another 10 mM when the released already treated solution was put to secondary degradation. The whole content of fumaronitrile degraded for 6 hours was 36 mM.

Conclusions

Inorganic-organic hybrid amorphous nanocomposites containing carrageenan are obtained via sol-gel method at room temperature. The FT-IR results suggest that in the samples containing MTES, strong chemical bonds between inorganic and organic parts of synthesized materials has been proved. The presence of a hybrid nanostructure with well-defined nanounits and their aggregates, formed by self-organizing processes, is clearly observed by the AFM studies.

The obtained results are a proof for the possibility of using a column bioreactor with biocatalysts obtained on the basis of TEOS-carrageenan hybrids with entrapped thermophilic cells, producers of thermostable nitrilase for a biodegradation process of toxic organocyanide substances. The obtained thermostable biocatalysts possess an increased tolerance towards nitriles and are suitable for biological treatment of hot waste waters from different industries, containing compounds with cyano-groups which is ecologically important too.

Acknowledgement

This work was supported by Grant NP 202 of the National Fund for Scientific Research, Republic of Bulgaria.

Bibliography

1. Carturan G. *et al.* (1998) *Production of Valuable Drugs from Plant Cells Immobilized by Hybrid Sol-Gel SiO₂*. Journal of Sol-Gel Science and Technology 13, 273-281.
2. Kang E. *et al.* (2004) *Modification of Thermo-Optic Characteristics of Sol-Gel Inorganic-Organic Hybrid Materials*. Journal of Sol-Gel Science and Technology 32, 277-280.
3. Ogoshi T. *et al.* (2005) *Organic-inorganic polymer hybrids prepared by the sol-gel method*, Comp. Int. 8-9, 539-566.
4. Hamano Y. *et al.* (2004) *Preparation of flexible inorganic-organic hybrid proton-conducting membranes via sol-gel processing*. Journal of Materials Science 39, 7097-7099
5. Schubert U. *et al.* (2003) *Silica-Based and Transition Metal-Based Inorganic-Organic Hybrid Materials-A Comparison*. Journal of Sol-Gel Science and Technology 26, 47-55.
6. Shchipunov Y. *et al.* (2005) *Hybrid organic-inorganic nanocomposites fabricated with a novel biocompatible precursor using sol-gel processing*. Composite Interfaces 11, 587-607.
7. Chen J. *et al.* (2004) *Preparation and characterization of porous hollow silica nanoparticles for drug delivery application*. Biomaterials 25, 723-727.
8. Sgarbi N. *et al.* (2004) *Self-assembled extracellular matrix protein networks by microcontact printing*. Biomaterials 25, 1349-1353
9. Tan X. *et al.* (2005) *An amperometric cholesterol biosensor based on multiwalled carbon nanotubes and organically modified sol-gel/chitosan hybrid composite film*. Analytical Biochemistry 337, 111-120.
10. Shchipunov Y. *et al.* (2005) *Polysaccharides as a template for silicate generated by sol-gel processes*. Journal of Colloid and Interface Science 285, 574-580.
11. Valle K. *et al.* (2006) *Hierarchically structured transparent hybrid membranes by in situ growth of mesostructured organosilica in host polymer*. Natural Materials 107-111.
12. Chen T. *et al.* (2003) *Enzyme-catalysed gel formation of gelatin and chitosan: potential for in situ application*, Biomaterials 24, 2831-2841.
13. Gill I. *et al.* (2000) *Bioencapsulation within synthetic polymers (Part 2): non-sol-gel protein-polymer biocomposites*. Tibtech 18, 469-480
14. Emanuilova E. *et al.* (2003) *Method for Degradation of Nitrile Compounds*. Official Bulletin of Republic of Bulgaria, Patent №12P13/02.
15. Chernev G. *et al.* (2005) *Silica nanocomposites containing polyacrilamide gel*. Glass Sci. & Tech. 179-184.
16. Chernev G. *et al.* (2005) *Silica hybrid nanocomposites*. CESJ 4 (1) (2006) 81-91. anotech., eds. Balabanova E, Dragieva I, Heron Press, Sofia 94-97.