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PRELIMINARY STUDY FOR USING VINYLTRIACETOXYSILANE AS PRECURSOR IN ENZYME IMMOBILIZATION BASED ON SOL-GEL METHOD

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SUMMARY

During the last years, sol-gel technology has become a well-established method for the preparation of catalytic active monoliths, bulk, particles and thin films. One reason for the increasing research activities in this field is the opportunity to obtain versatile hybrid materials by incorporation of different molecules, like dyes, enzymes, whole cells, chemicals and drugs. The aim of this research was to evaluate the suitability of vinyltriacetoxysilane (VTAS) as precursor in sol-gel enzyme immobilization and the physicochemical characterization of the final products (silica xerogels).

Keywords: sol-gel, protease, immobilization, physicochemical characterization, vinyltriacetoxysilane

INTRODUCTION

Interdisciplinary knowledge is becoming more and more important to circumvent the newly emerging problems in medical, environmental and industrial sectors [1].

Practical use of enzymes has been realized in various industrial processes and products including detergents, and is being expanded in new fields: fine-chemical synthesis, pharmaceuticals, biosensors, bioremediation, bioleaching, polymerase chain reaction, protein digestion in proteomic analysis, and biofuel cells. The specificities of enzyme catalysts promise improvements in many applications, but the short lifetimes of enzymes presently limit their usefulness [2, 3].

There have been many approaches to improve the enzyme stability: enzyme immobilization, enzyme modification, protein engineering, and medium engineering.

Enzyme immobilization represents the attachment or incorporation of enzyme molecules onto or into large structures, via simple adsorption, covalent attachment, or encapsulation [4-6].

Retention of the biologically active species is achieved by entrapping them in the porous matrix that is created during sol–gel formation. The relatively mild processing conditions, i.e. ambient temperature and use of pH-buffered systems, have led to a large number of sol–gel immobilized enzyme preparations. The entrapment of enzymes in a porous silica sol–gel has attracted a great deal of interest because of its potential use in industry [7-11].

The first and most important step in sol-gel synthesis is the selection of precursor, step that has a decisive effect on the final product morphology.

In present work we used a less known precursor vinyltriacetoxysilane (VTAS) in mixture with tetraethoxysilane (TEOS) and the result seems to be promising. Classical physicochemical characterization method like N_2 adsorption/desorption isotherms, FT-IR spectroscopy, thermal analysis, X-ray diffraction and transmission electron microscopy (TEM), were used. Different morphologies were obtained with various molar ratios of precursors.

MATERIALS AND METHODS

Materials: Alcalase (produced by *Bacillus licheniformis*), Folin-Ciocalteu's phenol reagent, L-Tyr and trichloroacetic acid were from Merck, Hammerstein casein, tetraethoxysiliane (TEOS), methytriethoxysilane (MTES), vinyltriacetoxysilane (VTAS) were from Fluka. All the other chemicals were commercially available reagents grade products and were used without purification.

Immobilization method: In the first step silica sol was obtained from a mixture of alcoxid, ethanol and water in acid catalysis (MTES or VTAS and TEOS / solvent / water / HCl = 1 / 0.8 / 1 / 0.004). In the second step, the gels are obtained from sol, enzymatic solution and catalyst (NaF). After gelation the gels were left for aging and drying.

Biochemical investigation: Proteolytic activity of immobilized Alcalase was determined at 37 ºC in phosphate buffer, pH 8, using Hammerstein casein as substrate. One unit of protease was equivalent to the amount of enzyme required to release 1 μmol of tyrosine/ml/min. The protein content of the immobilized protease was determined by the Lowry method using Folin Ciocalteu`s reagent and a standard calibration curve of bovine serum albumin.

Characterization of samples: Specific surface areas were calculated from N_2 adsorption isotherms measured on a Quantachrome NOVA 2000 instrument using the multi-point BET method. The total pore volume (V_p) was calculated in the last point of adsorption

branch. Pore size distribution curves were calculated from the adsorption (D_p [Ad]) and desorption (D_p [Ds]) branch of the isotherms using the Barrett– Joyner–Halenda (BJH) method. Infrared spectra were recorded on a Mattson Genesis 1 FT-IR spectrometer in 0.1 wt.-% KBr pellets. XRD patterns of each sample were recorded. X-ray diffraction patterns were run on a DRON 3 Russian made diffractometer operated under computer control. TEM micrographs were recorded on a Phillips CM 10 instrument using copper mounted holey carbon grids.

RESULTS AND DISCUSSION

In the sol-gel process, several parameters influence the final product physicochemical properties and the encapsulted protease activity. The precursor selection is an important part of sol-gel method. In this case, silica xerogels were synthetised using a mixture of TEOS and MTES or VTAS. In table I, the influence of different precursors in Alcalase activity and the xerogels physicochemical properties are presented. Various molar ratios of MTES/TEOS (MTT) and VTAS/TEOS (VTT) were used. Alcalase activity increased with increasing of MTES or VTAS ratio. By increasing the quantity of VTAS, in the mixture of VTAS/TEOS (VTT), the porosity of the xerogels decreased and the protease activity increased. The Alcalase activity decreased in the series: $activity_{MTT}$>$activity_{TEOS}$>$activity_{VTT}$. When VTAS/TEOS mixtures were used as precursor the Alcalase activity was lower, the possible reason is the release of acetic acid in the hydrolysis step. The acetic acid unfolds the enzyme. The best result is obtained in the case of MTES / TEOS 1:1 (MTT 1:1).

Table I. Precursor influence on the Alcalase activity and silica xerogel physicochemical properties

Name	Protease activity (U/g)	D_p [Ad] (Å)	D_p [Ds] (Å)	S_{BET} (m^3/g)	V_p (cm^3/g)
MTT 1:3	0.23	56.66	45.43	132.1	0.21
MTT 1:2	0.30	65.29	49.39	271.1	0.31
MTT 1:1	2.08	65.06	45.32	96	0.16
VTT 1:3	0.05	48.75	45.4	209.5	0.33
VTT 1:2	0.31	111.39	113.18	80.6	0.28
VTT 1:1	0.40	41.11	38.8	145.2	0.23
TEOS	0.47	232.66	180.08	71.5	0.45

All samples gave very similar IR spectra (Figure 1), with only the expected variations in band intensity. Presence of adsorbed water and free surface silanol groups as

well as siloxane linkages can easily be conceived from the IR spectra of silica in the range 400–4000 cm^{-1}. In the spectra the broad band located in the range 3000–3800 cm^{-1} corresponds to the fundamental stretching vibration of different hydroxyl groups. The broadness of this band suggests different local environments of the OH groups. The band at 1646 cm^{-1} is assigned to the deformation mode of water molecules, which are probably trapped inside the pores. In the range 400–1500 cm^{-1} the spectrum has several bands. Those located at about 461, 797 and 1090 cm^{-1} are the bond rocking, bond bending and bond stretching vibrations of the Si–O–Si units. Absence of band about 1725 cm^{-1} (characteristic band for carboxyl group) proves that hydrolysis reaction is finished.

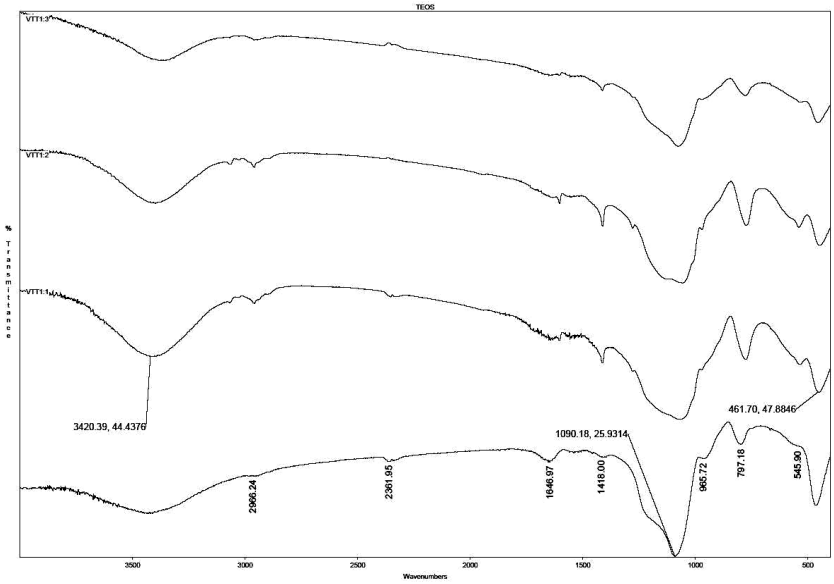


Figure 1. IR spectra of different molar ratio of VTT containing immobilized Alcalase

The thermogravimetric analysis indicates a multiple step weight loss: a small weight loss until 200°C – counting for the elimination of the adsorbed water molecules; a substantial weight loss from 200°C-500°C associated with an exotermic peak – attributed primarily to the removal of water by dehydroxylation and some loss of organic constituents (C,H,O); after 500°C the weight loss can occur due to the final dehydroxylation reactions and definitive carbonization of organic compounds, including the enzyme. The total weight loss is about 35%.

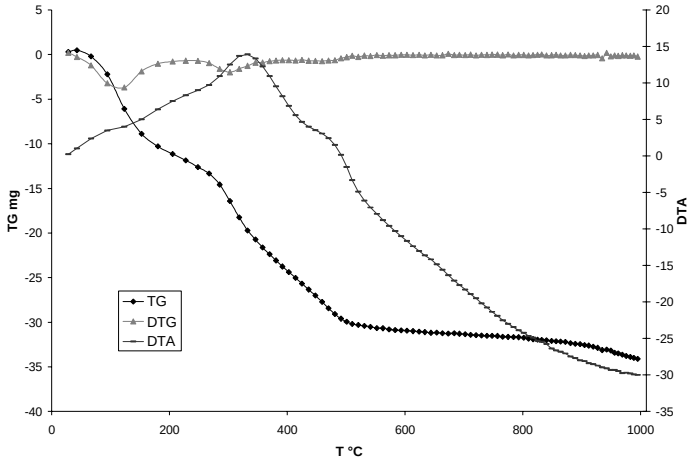
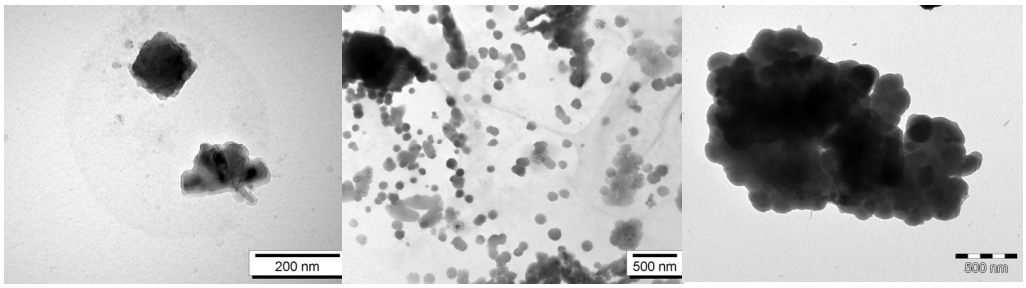


Figure 2. Thermal analysis of VTT 1:1

The X-ray diffractograms confirmed the amorphous structure of the xerogels. For more information TEM micrographs were recorded (figure 3). TEM micrographs show the structural modification of the silica xerogel when the precursors or the precursors' molar ratios are changed. In case of using TEOS as precursor big particles with lamellar structure were obtained, that is in concordance with the literature data. In case of VTAS/TEOS precursors, smaller particles were obtained and their morphologies are changed with the molar ratio.



VTT1:1 VTT1:2 TEOS

Figure 3. TEM micrographs of different mesoporous silica xerogels

CONCLUSION

The mixtures of VTAS/TEOS were successfully used in synthesis of silica xerogel with protease activity. The limitation of the process is the low protease activity caused by acidic pH, but that limitation could be an advantage when the optimal pH of the enzymes is in that range.

Physicochemical characteristics of the xerogels revealed potential utilization in sol-gel enzyme immobilization.

Diverse forms of xerogel particles are obtained with modification of the precursors' molar ratios, which preview a potential utilization as catalyst supports in different industrial processes.

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