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Adsorption of Chitosan into SiO_2 Monoliths Materials: Physical and Chemical Properties

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Summary: The dispersion of biopolymer chitosan on silica polymer matrix, prepared by the sol-gel method under conditions of nearly linear chain growth, has allowed us obtain the beneficial properties of the large surface area of the dispersed SiO_2 porous substrates. This study primarily aims to explore the chitosan adsorption in different weight ratios on silica monoliths by means solvent-aided dispersion. The chitosan on SiO_2 system has been investigated to know certain polymer properties, such as morphology, structure and thermal stability. The surface of the SiO_2 monolith disks calcined (500 °C) employed as matrixes for chitosan were fractures free. SBET surfaces areas of these monoliths range from 452.99 m^2/g. According to the FTIR analysis, the hydrogen bond there is an attractive intermolecular force between chitosan and SiO_2. In contrast, the amino groups of the biopolymer are still accessible as active sites for heterogeneous catalysis. In addition, the thermostability of chitosan deposited on the monoliths was better than chitosan isolate.

Keywords: adsorption; chitosan; monoliths; SiO_2

Introduction

In the last years, the sol-gel is an important technology for the development of new materials due to his versatility to obtain fibers, monoliths, coverings, microspheres and nanospheres.[1–3]

The sol-gel technique, in which glasses are formed from organic precursors by low temperature polymerization reactions, offers attractive advantages in terms of flexibility of composition and structure. Essentially the sol-gel process consists of three steps: first, a colloidal suspension of oxide particles (the sol) is formed by hydrolysis and condensation of an alkoxide precursor; then the sol is dried such that further condensation creates a semi-rigid 'gel'; finally, heat treatment of the gel is used to eliminate remaining organic ligands. The method has been widely studied for the fabrication of both bulk glasses and films,[1,2] the latter being formed typically by dip or spin coating of the sol.[4]

The precursors for synthesis of SiO_2 are alkoxysilanes such as tetraethoxysilane (TEOS),[5,6] and the most used technology is sol–gel, which provides an excellent way of obtaining transparent and mechanically stable films, bulk glasses and powders and porous materials.[7]

These materials have been used to applications as enzymatic immobilizations, catalysis, sensing, and affinity chromatography.[9–19]

It is quite a nontrivial result that sol-gel-entrapped enzymes should show any reac-

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tivity at all. These due to the monomers employed are highly reactive chemicals (alkoxides of semimetals and metals) capable of altering protein surfaces, and taking into account that the sol-gel process involves considerable shrinkage, thus exerting pressure on the proteins. In addition, the encapsulated enzymes must be oriented so that their active site is easily accessible to the pore network of the matrix.

We decide to experiment in that direction developing sol-gel monoliths impregnated with chitosan (CS). The chitosan used is in powder form, and is a partially deacetylated polymer of acetyl glucosamine, it is essentially a natural, water-soluble derivative of cellulose with unique properties. CS has been proved to be non-cytotoxic and having some biological activities.[21,22]

CS-based materials have aroused much interest in biomedical field.[23,24] One of the most important applications of the CS is the enzymatic immobilization. There are numerous reports about CS–enzyme conjugate, in order to make functional biomaterials with novel physiochemical and biological properties.[6,7]

SiO_2 and CS are material with important benefits; the CS can provide biological elements necessary for the enzymes and SiO_2 provides a rigid support and protection systems. This study proposes to explore the chitosan adsorption in different weight ratios into silica monoliths and test the relevant physical and chemical properties.

Experimental Part

Materials

Tetraethoxysilane (TEOS), Nitric acid, ethylic alcohol, Glacial acetic acid (AcOH) were purchased from Sigma–Aldrich Chemical Co., USA.

For CS, the degree of deacetylation is ca. 87% determined by a titration method and the average molecular weight (Mv) is 3×10^5 g/mol determined by viscosimetry in 0.1 M acetic acid and 0.2 M sodium chloride solution with constant temperature (25 °C).

CS was purchased from Sigma–Aldrich Chemical Co., USA and was used as received.

Synthesis of SiO_2 Monoliths

The SiO_2 monoliths were obtained by the sol-gel process, briefly, TEOS, H_2O and ethylic alcohol were mixed together and stirred for 1 h at 60 ± 1 °C yielding the hydrolyzed solution. The reagents molar ratio TEOS: H_2O: ethylic alcohol was 1/16/4 and 0.05 ml de HNO_3 was added to catalyze the condensation of silica.[8]

The principal chemical reactions involved in the sol-gel processing of inorganic materials are given below as Scheme 1. This scheme, consider the hydrolysis (1) and initial condensation reactions (2 and 3) of Organo (alkoxy)silanes ($R\text{-}Si(OR)_3$).[9]

$$\equiv Si\text{-}OR + HOH \underset{Esteryfication}{\overset{Hydrolysis}{\rightleftharpoons}} \equiv Si\text{-}OH + ROH \quad (1)$$

$$\equiv Si\text{-}OH + HO\text{-}Si\equiv \underset{Hydrolysis}{\overset{Water\ condensation}{\rightleftharpoons}} \equiv Si\text{-}O\text{-}Si\equiv + HOH \quad (2)$$

$$\equiv Si\text{-}OH + RO\text{-}Si\equiv \underset{Alcoholysis}{\overset{Alcohol\ condensation}{\rightleftharpoons}} \equiv Si\text{-}O\text{-}Si\equiv + ROH \quad (3)$$

The sol-gel solution was gelled into a mold to obtain disk monoliths with approximately 2 cm of diameter and kept at room temperature for 12 days. The gel samples were dried to a controlled temperature program from 18 °C until 100 °C by 14 h in an isotemp vacuum oven 282,[14] in order to minimize cracking.

The calcination of the sample was conducted under an air atmosphere in a muffle furnace at 500 °C for 6 h at a rate of 5.5 °C/ min.

Preparations of CS/SiO_2 Substrates

CS were dissolved in 100 mL of AcOH 0.2 M and five different CS concentrations, (0, 0.25, 0.5, 0.75 y 1 g) were used to evaluate the effects of adsorption of chitosan on SiO_2 monoliths. The SiO_2 monoliths were immersed in aliquots of 10 mL of AcOH solutions for 120 minutes, the solutions were maintained at 25 °C, 50 °C and 70 °C. After that, each monolith was removed and dried at 80 °C for 36 hours hrs, in an isotemp model 282A vacuum oven.

Analysis and Measurement

Viscosity of CS-AcOH solutions were analyzed using a stabinger viscometer SVM 3000.

A scanning electron microscope (SEM) model XLDESEM (Phillips) provided with X ray microanalyser, was used to observe the surface topography and fracture surfaces of monoliths.

The nitrogen sorption isotherm was obtained by static volumetric method at 196 °C. The specific surface area (m^2/g) was determined using BET (Braunauer, Emmet and Teller) method.

X-ray diffraction measurements were performed in a Rigaku D/max-2100 diffractometer (Cu Kα radiation) at 30 kV and 16 mA. The angular resolution was of 0.02°.

Structural analyses of the CS/SiO_2 were performed using a Nicolet 5ZDA spectrometer in the 4000 to 400 cm^{-1} range at a resolution of 4 cm^{-1} in transmission mode. All samples were prepared in FT-IR-grade KBr (Aldrich) to form pellets of 12.8 mm in diameter.

Thermogravimetric analysis (TGA) was performed on a Mettler TGA/STDA 851. The measurements were performed in the temperature range 0–450 °C (heating rate10 °C/1 min) in an argon atmosphere (50 ml/min). Samples were placed in aluminum pans (21 mg).

Results and Discussion

Viscosity CS-AcOH Solution

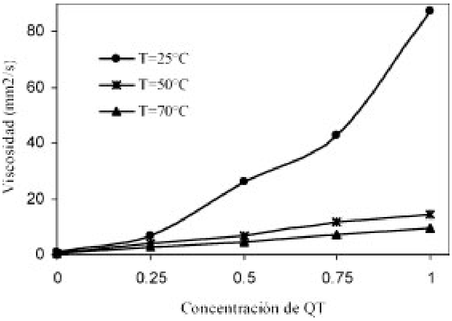

Figure 1.
Viscosity of solution with 0.25, 0.5, 0.75 and 1 g of CS.

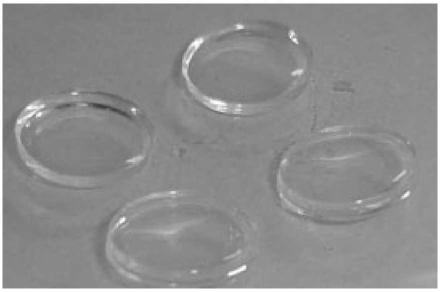

Figure 2.
Image of CS/SiO_2 monoliths.

Figure 1 show the viscosity of CS-AcOH solutions with different concentrations of CS. In the solutions at low temperature the viscosity increases, values were obtained until 87.4 mm^2/s. These is due to CS has many glucosamine groups that make it a polyelectrolyte biopolymer at pH acids.[25] Furthermore, the effect of CS concentration on the viscosity is much greater at low temperature

The CS/SiO_2 monoliths (Figure 2), obtained are transparent with morphologies well defined and do not exhibit irregularities.

The immersed monoliths with the maximum temperatures of 50 °C and 70 °C

Table 1.
Absorption of CS Monolith behavior, using a residence time of 120 min in CS-AcOH solutions.

grs of CS contained in a CS-AcOH solution	Temperature °C		
	% Adsorción de CS		
	25 °C	50 °C	70 °C
0.25	4.85	5.67	7.37
0.5	4.45	6.34	7.74
0.75	1.12	5.44	7.12
1.0	0.78	10.09	9.33

showed the highest percent absorption of CS (Table 1); which it is attributed to diffusion process facilitated by a decrease of solution viscosity, even in presence of a increase of CS concentration.

Morphological Analysis

The Figure 3 corresponds to the SEM surface of a SiO_2 monolith while the Figure 4 corresponds to the monolith surface of a CS/SiO_2. A comparative analysis of these surfaces shows a SiO_2 monolith with more uniform surface that CS/SiO_2. As illustration, the CS/SiO_2 monoliths obtained to 50 °C/1gCS/120 min indicates different surface morphology with globular particles were attributed to CS. It is seen that particles are exfoliated from the surface of SiO_2, because appear on the monolith a modified surface morphology. Some of the particles are smaller than 1 µm, with a relatively broad size distribution.

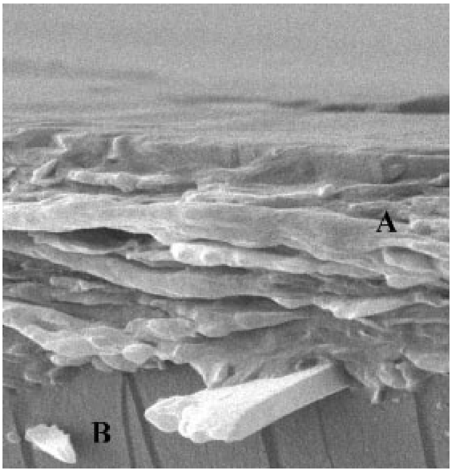

Figure 5.
SEM of transverse section of CS/SiO_2 monoliths obtained to 50 °C/1 gCS/120 min with an amplification of 6500X.

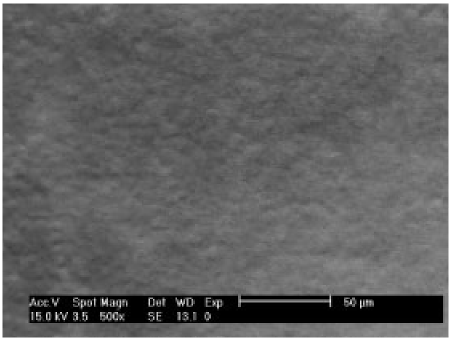

Figure 3.
SEM of surface morphology of SiO_2 monolith.

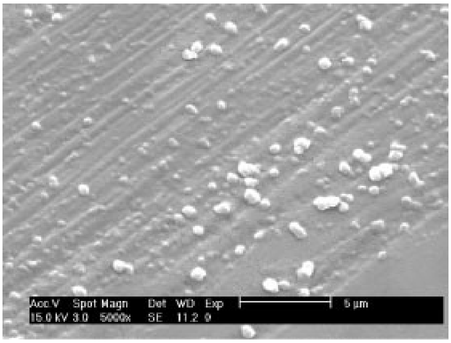

Figure 4.
SEM of surface morphology of CS/SiO_2 monoliths obtained to 50 °C/1 gCS/120 min.

Figure 5 show Scanning electron micrographs of transverse section of CS/SiO_2 monoliths. It is important to note there are two regions, **A** and **B**. In the region **A** was determined the major presence of layers deposited on silica bodies, which corresponding to CS. In the region **B** was observed a single phase. Through of chemical analysis to region **B** were determinated 58.9% of Silice, 29.5% of Carbon and 14.2% of Oxygen these confirm the adsorption process of CS into SiO_2. The morphological properties of the system described here point toward a structural ordering created by the molecular affinity among their components. Thereafter, noticeable differences between the properties of monoliths CS/SiO_2 and those of their parent components were expected. This indeed was observed when the thermal stabilities of the abovementioned types of monoliths were compared.

Gas adsorption measurements are widely used for determining surface areas of a variety of different materials. The measurements of adsorption at the gas/solid interface also form an essential part of many fundamental and applied investiga-

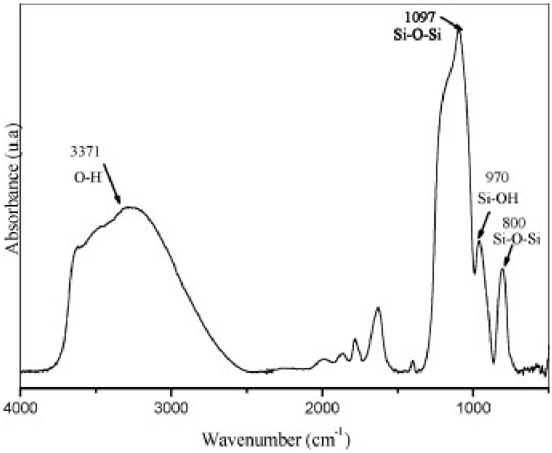

Figure 6.
FTIR of SiO_2 monoliths.

tions of the nature and behavior of solid surfaces.

The specific surface areas (SBET) of SiO_2 monoliths were 452.99 m^2/g. These characteristic offers several advantages for enzyme immobilization, a SBET between 300–1500 m^2/g is adapted for these processes.[10]

Structural Analysis

Figure 6 shows the Fourier transform infrared (FTIR) results of the SiO_2 monoliths, an intense peak at 1097 cm^{-1} can be assigned to Si−O−Si bond with stretching vibration and peak 970 cm^{-1} can be attributed to silanol groups (Si−OH).[12] Absorption at 800 cm^{-1} is due to the Si−O−C bond of siloxane groups.

Figure 7 shows that due to 87% deacetylation of chitosan, an absorption peak exist of C=O and CONH stretching vibrations at 1637 cm^{-1} and 1560 cm^{-1}. The absorption peak of 1160 cm^{-1} and 1080 cm^{-1} (symmetric ring vibrations) correspond to the

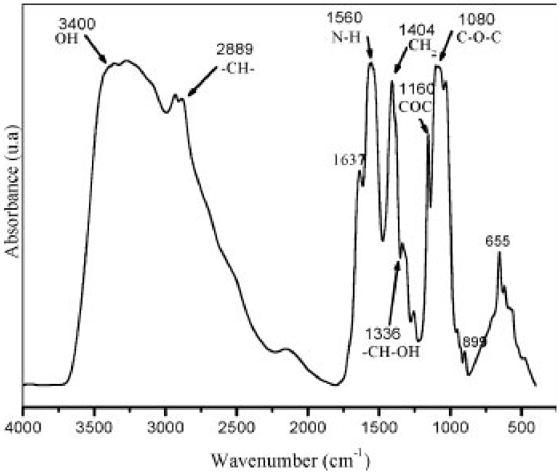

Figure 7.
FTIR of chitosan isolate.

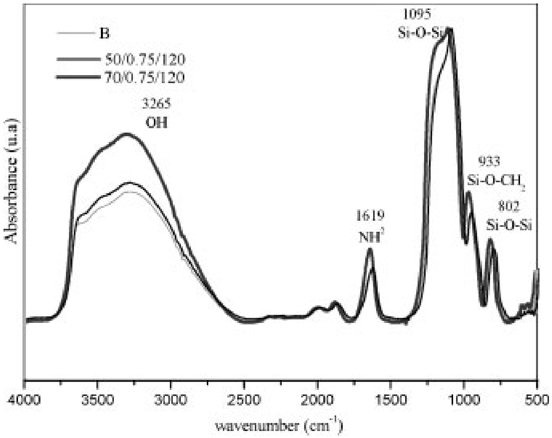

Figure 8.
FTIR of CS/SiO_2 monoliths obtained at (25 °C/1 g CS/120 min) (50 °C/1 g CS/120 min) (70 °C/1 g CS/120 min).

C–O–C bond, indicating the presence of chitosan.[12,26]

FTIR of CS/SiO_2 monoliths obtained at 25 °C, 50 °C, and 75 °C with 1 g of CS and immersion of 120 min; (25/1/120) (50/1/120) (70/1/120) are showed in the Figure 8. It is evident the absorption peak at 1619 cm^{-1} is due to CS. Note that original bands of CS of 1404 and 1160 cm^{-1} disappears in CS/SiO_2 monoliths.[27,28] It is observed a slight displacement in the position of absorption peaks of νs Si–O–Si at 1095 cm^{-1}.

A careful comparison of all spectra obtained of CS/SiO_2 monoliths varying the temperature (Figure 8) and CS concentration (Figure 9) indicates differences in the magnitude of the FTIR bands. The magnitudes of bands characteristics of CS (OH, NH_2 y $-CH_2$) were increases proportionally to temperature and CS concentration increase. In summary, these results add strong evidence that the temperature induce a greater adsorption of CS on the surface of SiO_2 monoliths.

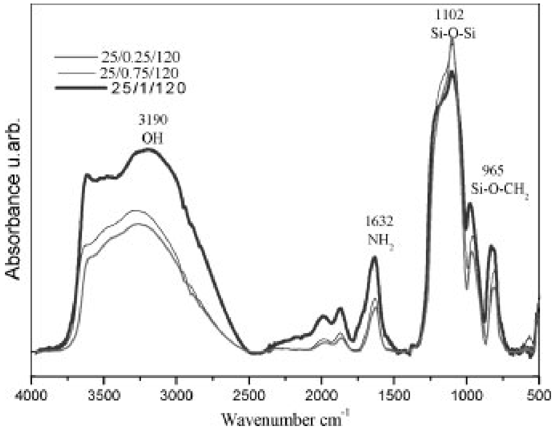

Figure 9.
FTIR of CS/SiO_2 monoliths obtained at (25 °C/0.25 g CS/120 min) (25 °C/0.75 g °CS/120 °min) (25 °C/1 g °CS/120 °min).

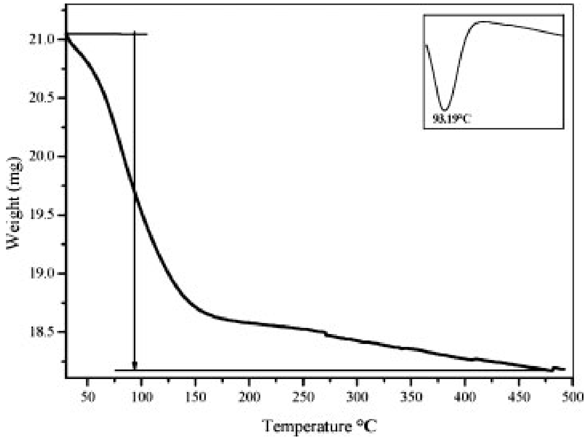

Figure 10.
TGA of CS/SiO_2 monoliths to 50 °C/1 g CS/120 min.

Thermal Properties

Figure 10 presents the TGA curve obtained of the CS/SiO_2 monoliths to 50 °C/1 g CS/120 min. The TGA curve displays two distinct temperature regions. In the zone 50–180 °C water physically adsorbed in the monoliths pores and on its surface undergoes evaporation. In the second region (180–470 °C) the thermal degradation of the organic residues takes place. The total weight loss of the CS/SiO_2 monoliths samples in the investigated thermal region (0–500 °C) shows the real mount of CS in the sample was about of 3.0 mg. This result indicates that CS has a lower thermal stability than CS pure, which is attributed to its high dispersion of CS on the SiO_2 monolith surface.

Conclusion

In this study, CS/SiO_2 monoliths were successfully prepared using an immersion process. The CS/SiO_2 had an excellent surface planarity and optical transparence, and the SiO_2 monoliths had a high CS content. A high CS content tended to form particles of CS which seem to be exfoliated from the surface of SiO_2, with a compact structure of the SiO_2 monolith. The FTIR results indices the CS content on SiO_2 monoliths increase proportionally with a increasing of temperature and CS concentration. The TGA results indicated that SiO_2 monoliths not serve as thermals protective substrates for CS. The excellent adsorption of CS by the SiO_2 monoliths provide the potential applications of the CS/SiO_2 monoliths as enzymatic immobilizations.

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