Chitosan–Organosilane Hybrids—Syntheses, Characterization, Copper Adsorption, and Enzyme Immobilization

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ABSTRACT: New organic–inorganic hybrids SiGCX (X = 1 to 3) were prepared from the biopolymer chitosan with a degree of the deacetylation of 86% and three distinct silylating agents of the type $(CH_3O)_3Si—R—NH_2$ [R = $—(CH_2)_3—$, $—(CH_2)_3NH(CH_2)_2—$ and $—(CH_2)_3NH(CH_2)_2NH(CH_2)_2—$]. Both chitosan and silylating agents have the amine groups crosslinking through linear glutaraldehyde units. Two stages were proposed for this synthetic method: crosslinking, and sol-gel processes. The resulting dried hydrogels are amorphous, insoluble in organic as well as acidic or alkaline aqueous media, and exhibited a lamellae-like surface morphology. The hybrids SiGCX (X = 2 and 3) have a larger adsorption capacity for copper ion than natural chitosan, with very similar kinetics of adsorption, defining a plateau after 1 h. The adsorption of copper increases with the organic chain length of the silylating agents: $[(1.72 \pm 0.05); (1.98 \pm 0.06)$ and $(2.49 \pm 0.07)] \times 10^{-2}$ mmol/g for SiGCX (X = 1 to 3), respectively, and chitosan adsorbed $(1.72 \pm 0.05) \times 10^{-2}$ mmol/g. These hybrids presented a good capacity for immobilizing enzymes, which decreased with the increase of the organic chain length of the silylating agents, that is, from SiGC3 to SiGC1. The amount of catalase immobilized for the hybrids SIGCX (X = 1 to 3) is 29.03 ± 0.87; 25.79 ± 0.77, and 17.94 ± 0.54 mg g^{-1}, respectively, which is larger than the value of 12.21 ± 0.37 mg g^{-1} obtained for chitosan. © 2000 John Wiley & Sons, Inc. J Appl Polym Sci 77: 797–804, 2000

Key words: glutaraldehyde; chitosan; crosslinking; sol-gel process; organic–inorganic hybrid

INTRODUCTION

Chitosan is currently prepared from the natural biopolymer chitin through its deacetylation in alkali medium. The original natural precursor chitin and the resulting biopolymer chitosan, are chemically similar to cellulose. However, the presence of the amine group $—NH_2$ on chitosan, at carbon-2 of the monomeric unit, drastically changes the properties by enhancing solubility and reactivity in relation to either chitin or cellulose.[1,2] These classes of biopolymers and some products obtained by their chemical modification have found multiple applications in various fields of investigations.[3]

Silylating agents of the general formula $(CH_3O)_3Si—R—NH_2$ present two reactive functional groups: the amine $—NH_2$, and methoxy $—OCH_3$ groups. Under the influence of specified amounts of water the latter group suffers hydrolysis to undergo polycondensation to finally form polysiloxane —SiOSi— groups through a sol-gel process. These silylating agents are well explored not only in many industrial applications, but also for the use in a lot of modifications of inorganic or

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organic matrix surfaces as well as to reinforce polymer systems with particulate inorganic fillers.[4] However, in recent years the sol-gel process has been revived as a new and interesting route to prepare inorganic–organic hybrids, which in fact, opened many new modes of preparing such kinds of composites.[5–7] In this connection, chitosan was also employed as a dispersant agent in the sol-gel process, characterizing a new method of immobilization of the enzyme α-amilase, without any interactive action.[8]

One important feature linked to the reactivity of chitosan is related to the availability of the amine group in condensing through an aldehyde function. For this purpose the linear dialdehyde chain of glutaraldehyde is largely explored due to its ability in detecting the presence of free amine groups in this biopolymer. This general procedure can be applied in many circumstances, embracing simple or complex inorganic and organic compounds.[9–14]

This present investigation reports the method of synthesis of the hybrids obtained by silylating agents and chitosan, whose final polymers are formed through the amino group crosslinking with glutaraldehyde. The results of some characterization and the use as an alternative approach to adsorption of copper cation and immobilization of enzymes by the obtained hybrids are also presented.

EXPERIMENTAL

Materials

Chitin from shrimps shells was acquired from Fine Chemical Kito (Palhoça-SC-Brasil). The silylating agents 3-(trimethoxysilyl)propylamine (TMA) (Aldrich), *N*-[3(tri-methoxysilyl)propyl]-ethylenediamine (TMD) (Aldrich), and -[3-(trimethoxysilyl)propyl]ethylenetriamine (TMT) (Aldrich) were reagent-grade products. Glutaraldehyde, 50% solution in water (Aldrich), urease from jack beans with 1.59 U mg^{-1} (Fluka), glucose oxidase from *Aspergillus niger* with 23.8 U mg^{-1} (Fluka), catalase from bovine liver with 47470 U mg^{-1} (Fluka), invertase from baker's yeast (*S. cerevisiae*) with 104.6 U mg^{-1} (Fluka), glacial acetic acid (Ecibra), EDTA (Nuclear), and copper nitrate (Vetec) and bidistilled water were used in all experiments.

Preparation of Chitosan

Chitosan was prepared by deacetylation of chitin in an alkali solution, 50% NaOH, for 1 h at 110°C. The solid was filtered and washed thoroughly with bidistilled water until nearly neutral pH. The solid was dried in vacuum at room temperature, and finally cut in a knife mill until sieved through at 80 mesh.

Preparation of Chitosan–Organosilane Hybrids

A recent prepared chitosan sample of 1.0 g (6.2×10^{-3} mol) was dissolved in 40.0 cm^3 of a 0.10 mol dm^{-3} of acetic acid solution. The expected insoluble fraction was separated by centrifugation. An equivalent amount of silylating agents (6.2×10^{-3} mol) was added to the dissolved chitosan. The mixture was mechanically homogenized for 1 h. Then, to this mixture 6.2×10^{-3} mol of glutaraldehyde per mol of nitrogen present on the silylating agents was added. A gel forms immediately and was kept standing for 24 h to complete the expected sol-gel process. After filtering, the gel was thoroughly washed with bidistilled water until the washing water was free of glutaraldehyde. Finally, the solid was dried in vacuum. All preparations were performed at room temperature, with the silylating agents TMA, TMD, and TMT, to give the hybrids named as SiGC1, SiGC2, and SiGC3, respectively.

Measurements

X-ray powder patterns were obtained with nickel-filtered Cu-Kα on a Shimadzu model XD3A diffractometer. Infrared spectra were obtained by using 1 wt % samples in KBr pellets with 20 scans and 4 cm^{-1} resolution, in a Bomem spectrometer series MB. ^{29}Si NMR spectra were obtained on a 300/p Bruker spectrometer with magic-angle spinning, operating in CP/MAS mode at 59.63 MHz with a pulse delay of 3 s and a contact time of 5 ms. The scanning electron micrographs were obtained from secondary electrons with a JEOL JSM-T300 microscope with EDS analysis. The concentration of the enzymes was determined with a UV-visible electronic equipment Beckman DU 640 spectrophotometer.

Degree of Deacetylation of the Chitosan

The degree of free amine groups —NH_2 on the chitosan has been determined by infrared spectroscopy.[15–17] This method is based on the relationship between absorbance (A) values at 1655 cm^{-1}, which is attributed to amide I, and the corresponding value of the hydroxyl band at 3450 cm^{-1}. In the present investigation the degree of deacetylation

(DD) was determined by applying the following equation[18]: DD = 97.67 − [26.486(A_{1655}/A_{3450})].

Copper Adsorption Capacity

Samples (20.0 mg) of chitosan or the hybrids SiGCX (X = 1 to 3), in a series of individual polyethylene flasks, were suspended in 10.0 cm^3 of 1.0×10^{-3} mol dm^{-3} of copper nitrate solution. The flasks were mechanically stirred at 298 ± 1 K for various intervals of time, varying from 10 to 180 min. After each defined time, the solid was separated by filtration. The amount of unsorbed cation was determined by titration of aliquots of the supernatant with a standard EDTA solution (1.0×10^{-2} mol dm^{-3}). The amount of copper adsorbed on surface was determined by means of the expression: $N_f = (N_i - N_s)/m$, where N_i is the initial number of mol of copper added, N_s is the number of mol of copper in solution after the equilibrium with the solid phase, and m is the mass of the biopolymer. Each experimental point was determined from duplicate runs.

Capacity for Enzyme Immobilization

Samples of chitosan and the hybrids SiGCX (X = 1 to 3) with a mass around 50 mg were suspended in 10.0 cm^3 of solution, containing 2.5 mg cm^{-3} of enzyme in phosphate buffer at pH 6.86 in closed glass flasks. The system was maintained in the mechanical stirrer for 2 h at 298 ± 1 K. In the immobilized process at solid/liquid interface the content of enzyme (E_{im}) was obtained by subtracting that determined in the supernatant (E_{fi}) from the initial amount (E_{in}) used for immobilization, by means of the expression: $E_{im} = E_{in} - E_{fi}$. The amount of free enzyme under equilibrium after immobilization was determined in the supernatant as previously described.[19]

RESULTS AND DISCUSSION

Degree of the Deacetylation of the Chitosan

Infrared spectroscopy is a useful and well-established tool to determine the degree of deacetylation of the chitosans.[15–17] The infrared spectra of chitosan and the hybrids of this biopolymers are shown in Figure 1. The absorbance ratio to be considered in this technique at 3450 and 1655 cm^{-1} was based on the obtained values 0.601 and 0.262, respectively. By applying these values in the corresponding equation,[18] DD = 86%, which corresponds to an acceptable degree of deacetylation for the chitosan biopolymer.

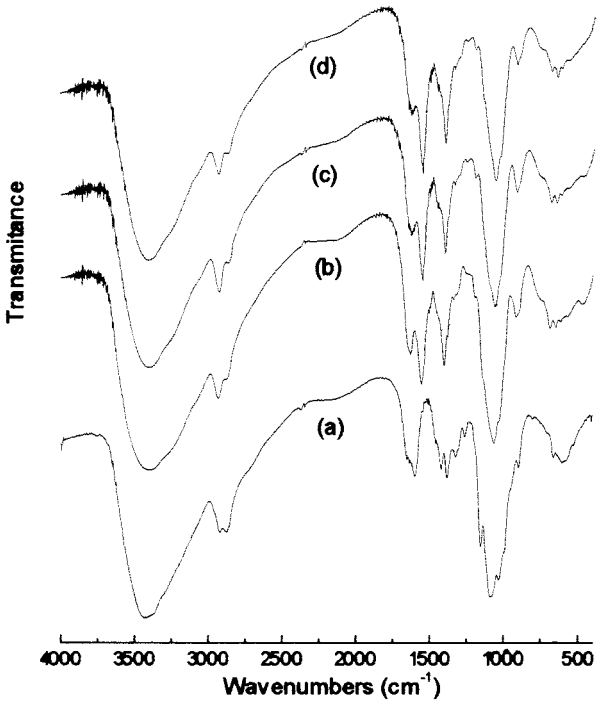


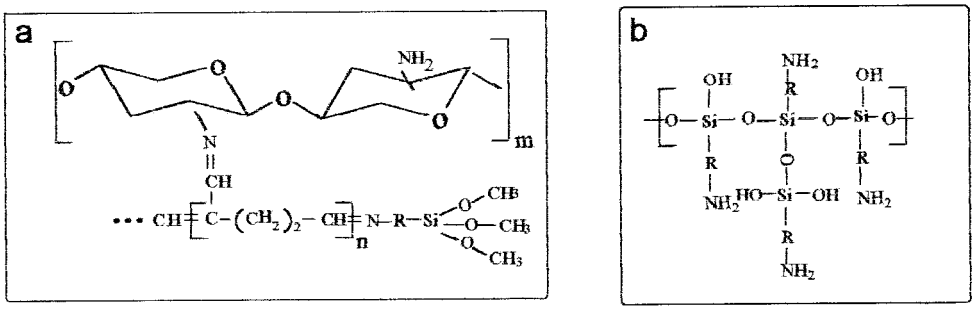
Figure 1 Infrared spectra of chitosan (a), SiGC1 (b), SiGC2 (c), and SiGC3 (d) hybrids.

Preparation of the Chitosan–Organosilane Hybrids

The main features of the synthesis of the hybrids involving chitosan and silylating agents may be divided into two distinct stages, which are illustrated in Scheme 1.

The first stage involves to the crosslinking process displayed in Scheme 1(a). The free amine —NH_2 group attached to the polymeric structure of chitosan and the identical functional group bonded to the end of the silylating agent chains are crosslinked by glutaraldehyde, through covalent interaction of both aldehyde —COH groups present at the ends of this linear dialdehyde molecule. From this interaction an expected stable imine bond is formed, due to the resonance established with adjacent double ethylenic bonds. As observed before, this crosslinking process forms a nonuniform chain length and terminal units of chitosan or organosilanes.[14]

In the second stage showed in Scheme 1(b), the sol-gel process takes place, forming a backbone of an inorganic polymer produced by hydrolysis and condensation of the methoxysilyl —$Si(OCH_3)_2$



Scheme 1. Probable mechanism of reaction of the hybrids: (a) crosslinking process, and (b) sol-gel process.

groups to yield the corresponding polysiloxane —SiOSi— inorganic chain.

Infrared Spectroscopy

Figure 1 shows the infrared spectra of the hybrids SiGCX (X = 1 to 3) with an identical set of bands, which are very similar in frequencies as well as in intensities. In comparing those spectra with that of the original biopolymer chitosan, it is clear that a new band appears at 1636 cm^{-1} for all hybrids. This band is attributed to the new imine N═C bond formed from the interaction of aldehyde and amine groups attached to the polymer, as expected for Schiff base formation. On the other hand, there is an increase in the intensity of the frequency of the band at 1560 cm^{-1}, attributed to the contribution of the ethylenic C═C bond formed from the resonance stabilization of the imine bond formed.[14] The same behavior was observed for the C—H stretching vibration frequency at 2932 cm^{-1}. In that case, the appearance of this band can be attributed to the increase of the contribution of glutaraldehyde and the silylating agent molecule on the final hybrids. However, there is no evidence of the characteristic band related to a free aldehydic group near 1720 cm^{-1} in any of the infrared spectra.[12,20] Therefore, these results are in agreement with the assumption of crosslinking as well as the absence of aldehyde groups in the final hybrids.

^{29}Si NMR Spectroscopy

The solid-state ^{29}Si NMR spectroscopy is a useful technique to elucidate the structures of the silicates, and the same kind of interpretation is applied to this series of hybrids. As a sol-gel process is involved, the appearance of these peaks is associated with the formation of the siloxane —SiOSi— and/or silanol —SiOH groups. Thus, an expected sequence of peaks can be related to the formation of the following new species, after concluding the reaction: (a) three siloxane groups bonded to silicon without any silanol group, as represented by the species $RSi(OSi)_3$, (b) two siloxane groups and one silanol group as indicated by —$RSi(OSi)_2(OH)$, (c) one siloxane group and two silanol groups, as formulated by —RSi(OSi)$(OH)_2$, and (d) absence of any siloxane linkages in the $RSi(OH)_3$ species with three free silanol groups, —$RSi(OH)_3$.[21–24]

Investigations on such similar compounds should present four main signals at −66; −58; −50, and −40 ppm identified in the final product.[21–24] These results are related to the degree of inorganic polymerization, which is reflected in the degree of polysiloxane group formation by the sol-gel process, according to the presence and intensity of the four distinct signals. The solid-state ^{29}Si NMR spectra of the hybrids are shown in Figure 2. The first three mentioned peaks are present in the spectra of the hybrids confirming the success of the sol-gel process in this kind of synthesis of hybrids, but with distinct extension of the reaction to form inorganic polysiloxane. The spectrum of SiGC1 shows a high intensity for the peaks at −66.0 and −58.7 ppm, and a low intensity for the one at −49.5 ppm, which confirms a large population of siloxane groups in this hybrid. The spectrum of SiGC2 shows peaks of medium intensity at −66.7 ppm, high and low intensities at −58.7 and −49.7 ppm, respectively, indicating the presence of a large population of siloxane groups in this hybrid as well, but in a lower extension when compared to SiGC1. The spectrum of SiGC3 shows peaks of medium intensity at −66.6 and −50.3 ppm, an intense peak at −58.5 ppm and low intensity of the peak at −39.7 ppm.

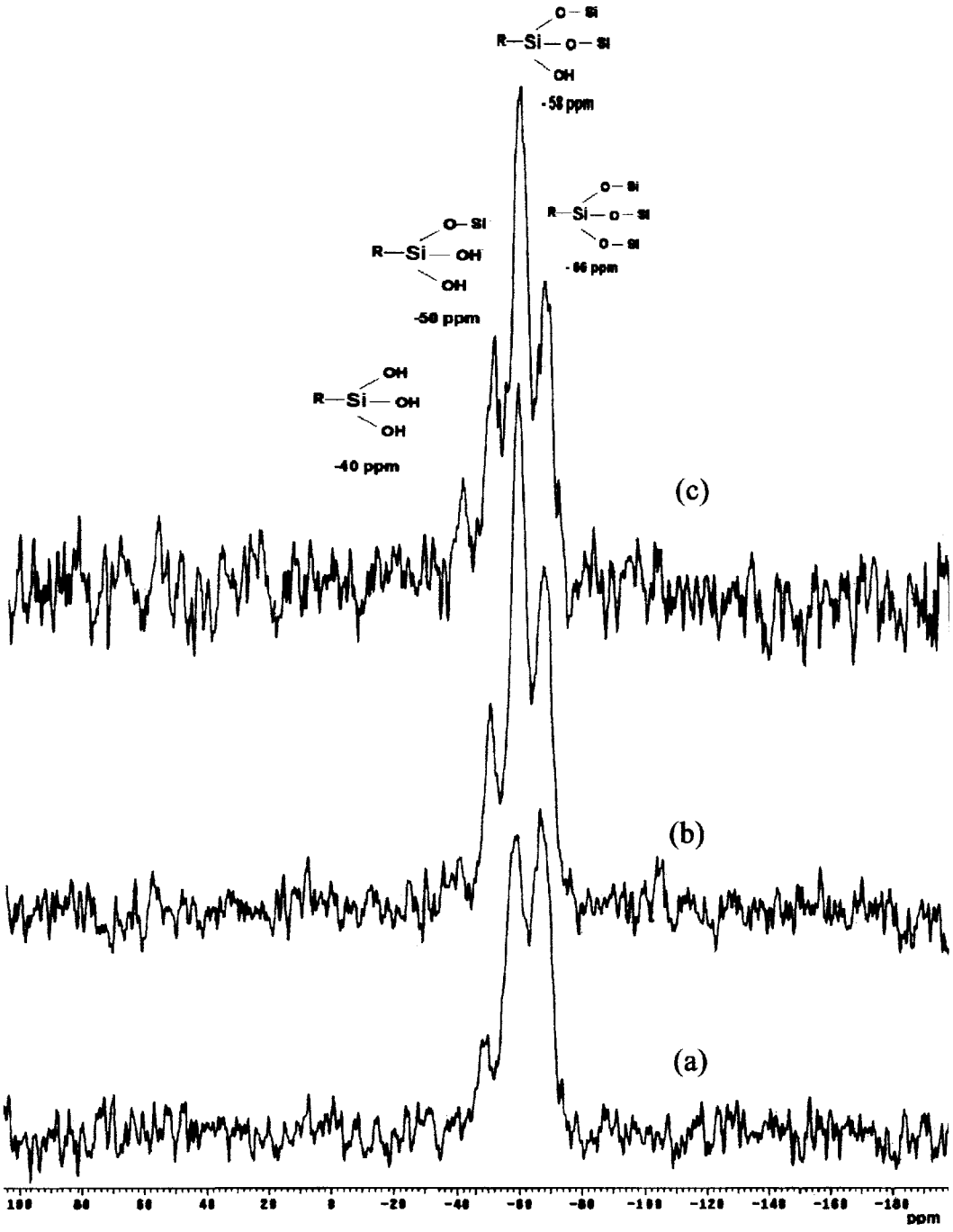


Figure 2 Solid state ^{29}Si NMR CP/MAS of SiGC1 (a), SiGC2 (b), and SiGC3 (c) hybrids.

This last peak confirmed the absence of siloxane groups from the silylating agent TMT (*N*-[3-(trimethoxysilyl)propyl] ethylenetriamine); therefore, this hybrid presents a smaller degree of polysiloxane formation than SiGC1 and SiGC2 hybrids.

The complete ^{29}Si NMR spectra data showed that all hybrids present a high degree of polysiloxane formation, and the degree of inorganic polymerization is reduced as the organic chain length of silylating agent increases. Therefore, in this reaction the increase of the organic chain length of the silylating agent suggests that a disturbance occurred in the to sol-gel process.

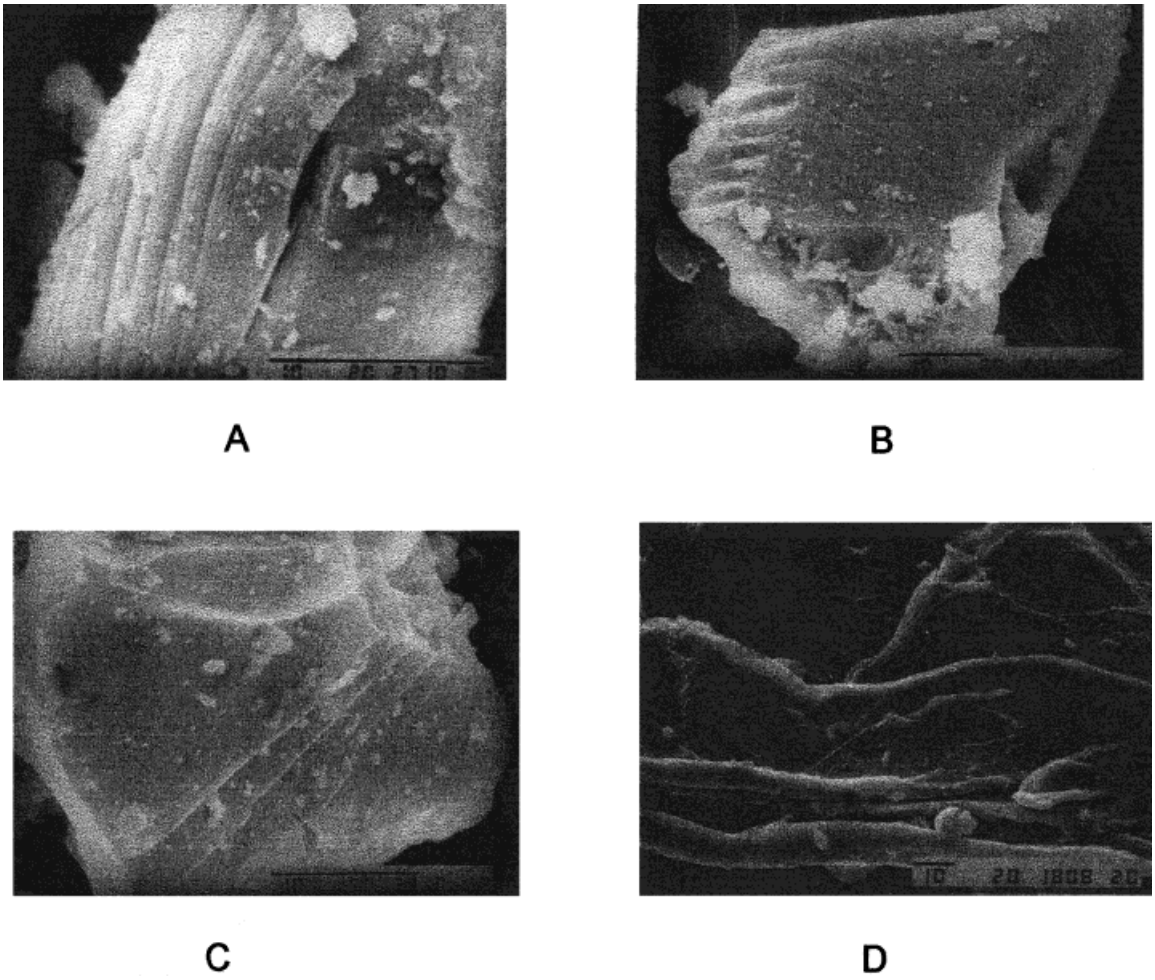


Figure 3 Scanning Electron Micrographs (SEM) of the fractured surface of SiGC1 (A), SiGC2 (B), SiGC3 (C) hybrids and chitosan (D).

Scanning Electron Microscopy

The morphology of the hybrids was investigated by the scanning electron microscopy (SEM) technique. The micrographs of the fractured surfaces of the hybrids are shown in Figure 3(A), (B), and (C), demonstrating their homogeneous morphology. This homogeneity is most probably due to the good miscibility of the chitosan and silylating agents. The fractured surface of the hybrids exhibited a lamella-like structure. This morphology is very different from that of the natural chitosan surface, as shown in Figure 3(D). The micrographs showed a small irregularity of the particles on the surface of all hybrids. This fact can be related to a consequence of immiscibility of the inorganic polymer from the sol-gel process formed prior to the crosslinking process.

Adsorption Capacity

In attempting to explore the present system, copper ion was chosen as a model to examine the adsorption capacity, due to the fact that this divalent ion is normally adsorbed by natural chitosan in an aqueous medium near neutral pH values.[25–27]

The isotherms of adsorption of chitosan and also of the hybrids are shown in Figure 4. By comparing chitosan with the SiGC1 hybrid, both kind of polymers presented identical adsorption capacity for copper cation of $(1.72 \pm 0.05) \times 10^{-2}$ mmol g^{-1}. The other hybrids showed larger capacity of adsorption than chitosan expressed as: $[(1.98 \pm 0.06)$ and $(2.49 \pm 0.07)] \times 10^{-2}$ mmol g^{-1} for SiGCX ($X = 2$ and 3). This behavior can be easily distinguished as indicated by error bars on the isotherms. The adsorption capacity among the

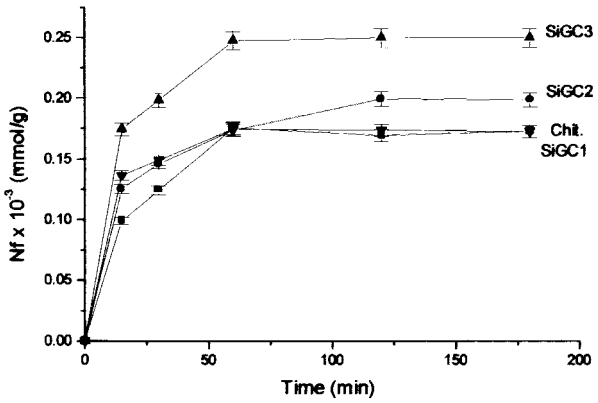


Figure 4 Batch isotherm of adsorption capacity of 0.10 mol dm^{-3} of copper solution at 298 ± 1 K for various intervals of time for SiGC1 (■), SiGC2 (●), SiGC3 (▲) hybrids, and chitosan (▼).

hybrids increases according to the increase of the organic chain length of the organofunctionalized silane used in the condensed polymer. On the other hand, all hybrids and chitosan showed a very similar kinetics of adsorption, defining a plateau after 1 h.

Capacity of Immobilization of Enzymes

The amount of four different enzymes supported on the chitosan and on hybrids was measured after 2 h of contact at 298 ± 1 K. These data, in mg of enzymes per gram of polymer are summarized in Figure 5. The immobilization results obtained from this study showed that the amount of immobilized catalase increased from chitosan to SiGC1 hybrid, but decreased from SiGC1 to SiGC3. The amount of immobilized glucose oxidase on chitosan is similar to that of the SiGC1 hybrid, which is equal to catalase, but decreased from the SiGC1 to the SiGC3 hybrid as well. The amount of immobilized invertase is similar for chitosan, SiGC1 and SIGC2 hybrids, but has a strong decrease to SiGC3 hybrid. The amount of immobilized urease is lower than that of chitosan for all hybrids, which are very similar to each other.

The interaction between some enzymes and modified chitosan after a reaction involving glutaraldehyde has been previously studied.[28] This investigation concluded that the interaction is mediated by hydrophobic as well as electrostatic interactions between charged enzyme surfaces and the free amino groups disposed on chitosan or on chitosan–organosilane hybrids. Another feature is related to the substantial increase in the binding of enzymes to chitosan modified by glutaraldehyde.

The participation of glutaraldehyde in bridging the organic and inorganic parts of the hybrids was expected to give an increase in the capacity of immobilization of enzymes on the hybrids, but this behavior was not proven in this investigation. The results indicated that the amount of immobilized enzymes decreases from SiGC1 to SIGC3 hybrids. This decrease in the amount of immobilized enzymes can probably be related to the difficulty of the macromolecules of the enzymes to access the hybrids particles, which is caused by increasing the crosslinking process on the SiGC1 to SiGC3 hybrids.

CONCLUSION

The prepared chitosan showed a good degree of the deacetylation with 86%. The synthesis of the hybrids involving chitosan, silylating agents, and glutaraldehyde may be divided into two stages: crosslinking, and sol-gel processes. The mechanism for both processes is proposed from the results of infrared and ^{29}Si NMR spectroscopic techniques.

The resulting hybrids presented a characteristic hydrogel state, while the dry forms showed an amorphous state, being insoluble in organic as well as acidic or alkaline media. The fractured surface of these hybrids, examined by SEM technique, exhibited a lamella-like morphology,

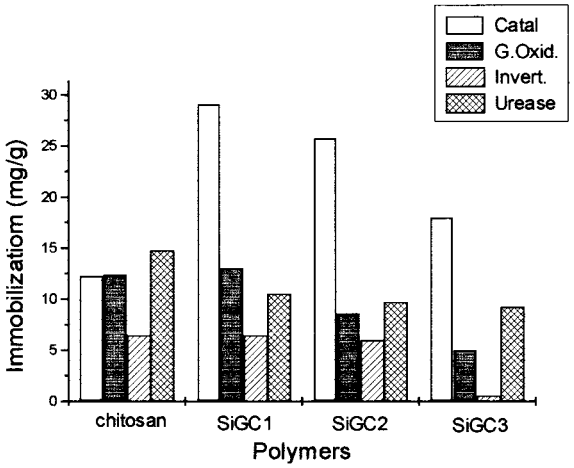


Figure 5 Batch isotherm of immobilization of the enzymes catalase, glucose oxidase, invertase, and urease on chitosan, SiGC1, SiGC2, and SiGC3 hybrids, measured after 2 h of contact at 298 ± 1 K.

strongly differing from that obtained for the original chitosan.

With the exception of SiGC1, all other hybrids displayed a larger adsorption capacity than chitosan. This capacity increased in agreement with the increase of the organic chain length of the silylating agent used in the synthetic route.

The hybrids presented a large capacity to anchor enzymes. With the exception of the catalase, the capacity for immobilization of enzyme was lower than chitosan. The adsorption of copper decreased with the increase of the organic chain length of the silylating agents. This fact can be probably attributed to the decrease on the accessibility to the hybrid particles of the enzyme macromolecules, caused by an increase in the crosslinking process with the SiGC1 to SiGC3 hybrids.

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