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Copper adsorption and enzyme immobilization on organosilane-glutaraldehyde hybrids as support

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Summary

New hybrids SiglutX (X=1 to 3) were synthesized from silylant agents: $(CH_3O)_3Si$-R-NH_2 [R = $-(CH_2)_2-$, $-(CH_2)_2NH(CH_2)_2-$ and $-(CH_2)_2NH(CH_2)_2NH(CH_2)_2-$]. The primary amine groups crosslinked with linear glutaraldehyde in two stages: crosslinking and sol-gel processes. The resulting polymers are amorphous, insoluble in organic as well as in acidic or alkaline aqueous media. The hybrids have a large capacity for copper adsorption with very similar kinetic behaviors, defining a plateau after 1 h. The adsorption increases with the increase of nitrogen atoms attached to the organic chain length of the precursor silylant, being 0.25±0.01, 0.37±0.01 and 0.50±0.01 mmol g^{-1} for X = 1 to 3, respectively. These hybrids also presented a good ability for immobilizing enzymes with distinguishable affinities. The amount of catalase and urease immobilization increased significantly from Siglut1 to Siglut2, while the amount of glucose oxidase and invertase anchored decreased from Siglut1 to Siglut2. Undefined amounts of enzyme immobilized for Siglut3 could be related to possible difficulties arising from its degradation during the interactive process.

Introduction

A simple inspection of the silylant agent molecules of the general formula $(CH_3O)_3Si$-R-NH_2 permits conclusions about the presence of two distinguishable reactive functional groups, the amine $-NH_2$ and methoxy $-OCH_3$ groups, disposing at the opposite ends of this important class of molecules [1,2]. In every case, under the influence of the presence of traces of water molecules, the latter groups are very active in undergoing hydrolysis to progressively react by polycondensation, to yield the polysiloxane (-SiOSi-) groups in the known classical sol-gel process [3]. A series of silylant agents, such as represented in the general formula, are well-explored not only in many industrial applications, but also for use in a lot of inorganic or organic matrix surface modifications. Normally, the covalent bond is attached to the inorganic support and this reaction is used to reinforce polymer systems with particulate inorganic fillers, among other applications. 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 kind of hybrids [4,5]. One important aspect linked to the reactivity of silylant agents the availability of the primary amine group for condensing through an aldehyde function. The linear dialdehyde chain, in particular glutaraldehyde, is greatly explored due to its ability to act bifunctionally in the detection of the presence of the original free amine groups,

that could be available in silylant agents [6], as explored in many circumstances to detect free basic organic functions in simple or complex inorganic and organic systems [6-8]. Clear applications of this reaction can be illustrated with many other substance that present the free amine group in its structure [9,10], which perform many catalytic reactions on inorganic organic hybrids [11] and enzymatic reactions on organic [12] or inorganic [13] supports. The present investigation reports a synthetic method directed to new hybrids obtained with silylant agents and glutaraldehyde and the use as copper adsorption and for enzymes immobilization.

Experimental

The silylant agents 3-(trimethoxysilyl)propylamine (TMA), N-[3(trimethoxysilyl)-propyl]-ethylenediamine (TMD) and [3-(trimethoxysilyl)-propyl]ethylenetriamine (TMT) were reagent grade products. Glutaraldehyde, 50% solution in water, urease from jack beans with 1.59 U mg^{-1}, glucose oxidase from Aspergillus niger with 23.8 U mg^{-1}, catalase from bovine liver with 47470 U mg^{-1}, invertase from baker's yeast (S. cerevisiae) with 104.6 U mg^{-1}, glacial acetic acid, EDTA (Nuclear), copper nitrate and doubly distilled water were used in all experiments.

The organosilane-glutaraldehyde hybrids were synthesized by adding an equivalent amount of glutaraldehyde in aqueous solution to $6.2x10^{-3}$ moles of each silylant agent. After mixing a dense solid immediately settled out, and allowed to stand for 24 h to complete the sol-gel process. The solid was thoroughly washed with doubly distilled water until the washing liquid was free of glutaraldehyde and, finally, the solid was dried in a vacuum. All preparations were performed at room temperature with TMA, TMD and TMT gave Siglut1, Siglut2 and Siglut3 hybrids, respectively.

X-ray powder patterns and infrared spectra were obtained as before [14]. ^{29}Si NMR spectra were obtained on a 300/P Bruker spectrometer with magic angle spinning, operating in the CP/MAS mode at 59.63 MHz with a pulse delay of 3 s and a contact time of 5 ms. The concentration of the enzymes was determined in a UV-visible Beckman DU 640 spectrophotometer by applying the Lowry method [15].

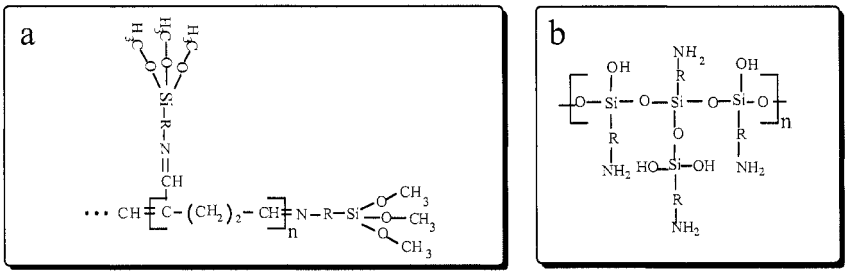
SiglutX (X=1 to 3) hybrid samples of 20.0 mg were suspended in a series of individual polyethylene flasks, containing 10.0 cm^3 of 1.0 x 10^{-3} mol dm^{-3} of copper nitrate solution, which were mechanically stirred at 298 ± 1 K for intervals of time, varying from 10 to 180 min and after each defined time, the solid was separated by filtration. The unsorbed cation amount was determined by titration of aliquots of the supernatant with EDTA solution of 1.0 x 10^{-2} mol dm^{-3}. The amount of copper adsorbed on the surface was determined by means of the expression: Nf = (Ni - Ns)/m, where Ni and Ns are the initial and final number of moles of copper the equilibrium in the solid/liquid interface, and m is the mass of the hybrids used in grams [16]. Each experimental point was determined in at least duplicate runs.

The capacity for enzyme immobilization was obtained by considering samples of hybrids SiglutX (X = 1 to 3) with a mass around 50 mg 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 mechanical stirrer for 2 h at 298 ± 1 K. In this immobilized process 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 [8], by means of the expression: $E_{im} = E_{in} - E_{fi}$. The amount of free

enzyme in equilibrium after immobilization was determined in the supernatant by employing Lowry method [15].

Results and discussion

The synthetic method involving the direct reaction between the silylant agent and glutaraldehyde immediately causes formation of a solid, which is favored by Shift base formation, with promptly manifestation in very strong exothermic reaction for all isolated hybrids. The proposed mechanism of these hybrids formation should be established in two distinct stages previously observed for similar interactions, by involving the same series of silylant agents with glutaraldehyde [8], as illustrated in Scheme 1.



Scheme 1. Probable mechanism of reaction to form the hybrids: a) crosslinking step caused by the reaction of glutaraldehyde with silylant agent and b) sol-gel process.

The crosslinking process displayed in the first stage in Scheme 1a, the free amine - NH_2 groups, bonded to the end of the silylant agent chains, are crosslinked with free glutaraldehyde reagent. Thus, the aminated function interacts with each aldehyde - COH groups of the linear dialdehyde molecule, causing the formation of covalent bonds. The stable imine bond formed, characterizing the Schiff base, is caused by the resonance established with adjacent double ethylenic bonds, which crosslinking process forms a non-uniform chain length, containing terminal organosilane units [8,17,18]. The second stage, shown in Scheme 1b, the sol-gel process takes place by forming the backbone of an inorganic polymer. This inorganic layer is produced through the hydrolysis and condensation of the methoxysilyl $-Si(OCH_3)_2$ groups, to yield the corresponding polysiloxane with infinite -SiOSi- chains. This stage is supposed to be much slower than the first one proposed for this mechanism and to complete the overall sol-gel process, was necessary to keep the reaction standing for a 24 h period.

The infrared spectra of all three hybrids SiGlutX (X = 1 to 3) are shown in Figure 1. Identical set of bands are very similar in frequencies, as well as in intensities. An important set of bands is attributed to the C-H stretching vibration frequencies appeared at 2936 and 2856 cm^{-1}. These bands correspond not only to silylant agent groups, but also to the contribution of glutaraldehyde in reacting with the silylant agent molecules during the final similar hybrids formation [17,18]. In case of the free aldehydic group, its characteristic vibrational band should be observed as a consequence of its partial reaction. Thus, the remaining free aldehyde groups would

show a signal near 1720 cm^{-1}. However, such a band was not detected in any infrared spectra. [6]. On the other hand, the band that appears with all hybrids at 1636 cm^{-1} is attributed to the new imine N=C bond formed from the interaction of the aldehyde and the amine groups attached to the silylant agent, as expected, due to Schiff base formation.[8,17,18].

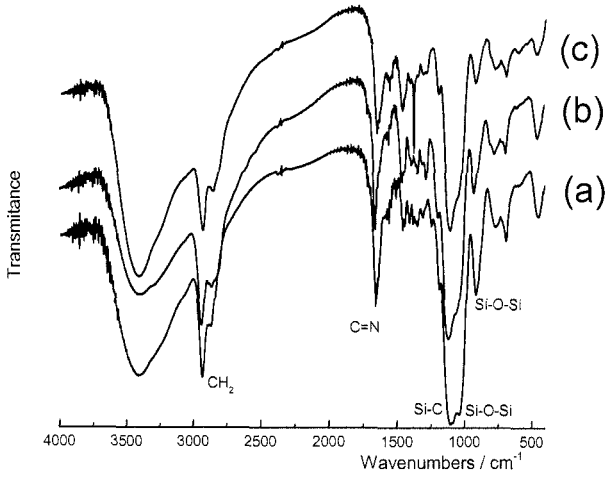


Figure 1. Infrared spectra of Siglut1 (a), Siglut2 (b) and Siglut3 (c) hybrids formed by the organosilane-glutaraldeyde interaction.

The band assigned to the contribution of the ethylenic C=C bond at 1560 cm^{-1} is formed by resonance stabilization of the imine bond. The band associated with C-Si bond appeared at 1118 cm^{-1} and for Si-O-Si bond the bands were attributed at 1038 and 914 cm^{-1}, confirming the presence of the silylant agents in the formation of the new hybrids [8]. Therefore, these results are in agreement with the assumption of crosslinking and the sol-gel process, as well as the use of all aldehyde groups of this interactive molecule in the final hybrids.

The final inorganic backbone proceeding from sol-gel process, causing by silylant agent hydrolysis is composed by silicon species such as siloxane, -SiOSi-, and/or silanol, -SiOH, groups. Then, an undoubtedly confirmation of these species in the solid state is established by ^{29}Si NMR spectroscopy, which is a useful technique to elucidate the structures of the silicates [19]. The appearance of a given number of peaks of peaks is associated with the formation of the new species: a) three siloxane groups bonded to silicon atom without any silanol group, $RSi(OSi)_3$ species, b) two siloxane groups and one silanol group $RSi(OSi)_2(OH)$, c) one siloxane group bonded with two silanol groups, $RSi(OSi)(OH)_2$ and d) without siloxane linkages, but containing three free silanol groups, $RSi(OH)_3$. Based on the product silylant agents hydrolysis, a set of signals are detected at - 66; - 58; -50 and - 40 ppm [19]. The sequence of peaks is related to the degree of inorganic polymerization, which reflected the degree of polysiloxane group formation during the sol-gel process. The presence and intensity of four distinct signals depends on the extension of the occurrence of the hydrolysis. The solid state ^{29}Si NMR spectra for all three hybrids are shown in Figure 2, and only the first two mentioned peaks for all hybrids were observed. This result confirmed the success of the sol-gel process in this kind of synthesis of hybrids,

indicating that the extent of the reaction which occurred in inorganic polysiloxane formation. These results are in agreement with a differentiated extension in the polysiloxane group formation in the sol-gel process with the three new hybrids. The Siglut1 hybrid showed a lesser intensity peak at - 66.0 ppm and a more intense one at - 58.7 ppm, data which confirmed a large population of siloxane groups. For Siglut2 the spectrum showed peaks of high and medium intensities at - 66.7 and - 58.7 ppm, respectively, indicating the presence of a large population of siloxane groups in this

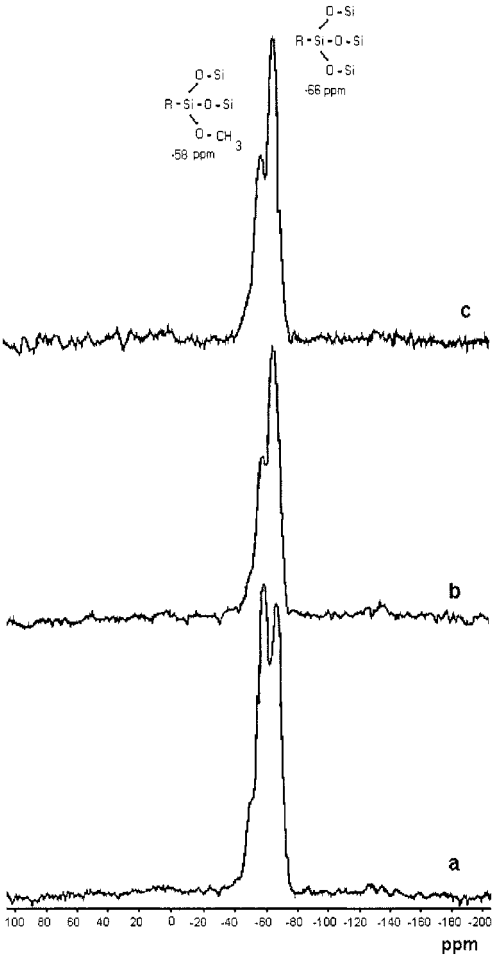


Figure 2. Solid state ^{29}Si NMR CP/MAS of Siglut1 (a), Siglut2 (b) and Siglut3 (c) hybrids.

hybrid as well, but in a larger extent, when compared to the preceding hybrid. The Silut2 and Silut3 hybrids presented similar spectra, with the absence of signals in the - 50 and – 40 ppm region. This fact confirmed the reduction of the $RSi(OH)_3$ and $RSi(OSi)(OH)_2$ species during the formation of such hybrids. The complete ^{29}Si NMR spectra data shown in Figure 2 confirmed that all hybrids presented a high degree of polysiloxane formation and the degree of inorganic polymerization is increased as the organic chain length of silylant agent increases. These results also are in agreement

that, in this series of reactions, with increasing the organic chain length of the silylating agent, a more favored sol-gel process occurred, fact which was not previously observed when chitosan hybrids were synthesized [17].
The organosilane/glutaraldeyde hybrids composed by equimolar amounts are expected to present basic center providing from amine free groups, which available to adsorb cations. Divalent copper was chosen as a model to examine this property, due to the fact that it is normally adsorbed by polymers modified with glutaraldehyde in an aqueous medium near biological pH values [18,20]. Thus, copper-chitosan complex formation had the thermodynamic data determined in aqueous solution [8] and chemically modified chitosans can be employed to adsorb metal ions from aqueous solution in variable pH values [21]. However, the present series of hybrids when exposed to aqueous cation solution, the extracted amount from batch procedure gave isotherms as shown in Figure 3. The hybrids demonstrated comparable capacities of adsorption with other polymers modified with glutaraldehyde [18,20]. For example, crosslinked chitosan-glutaraldyde compounds showed the adsorption capacities varying from 0.20 to 0.45 mmol g^{-1}, which values obtained are are larger than that obtained for unmodified chitosan, with 0.15 mmol g^{-1}. The organosilane/glutaraldeyde gave the sequence of values: 0.25 ± 0.01, 0.37 ± 0.01 and 0.50 ± 0.01 mmol g^{-1} for Siglut1, Siglut2 and Siglut3, respectively, being the last hybrid presented the highest adsorbed value. The adsorption capacity among the hybrids increases according to the increase of the organic chain length of the silylant agents used in the condensed polymer. This fact is easily understood because an increase amount of basic nitrogen atoms are progressively attached to the organic chain of the silylant agents used in the hybrid formation. This distinction among in each isotherm is reflected in the quantity of amino groups, that increases from one in TMA to three in TMT, with values varying from 0.25 to 0.50 mmol g^{-1}, in a very similar kinetics of adsorption, defining a well-formed plateau after only 1 h of exposing the cations to an individual hybrid.

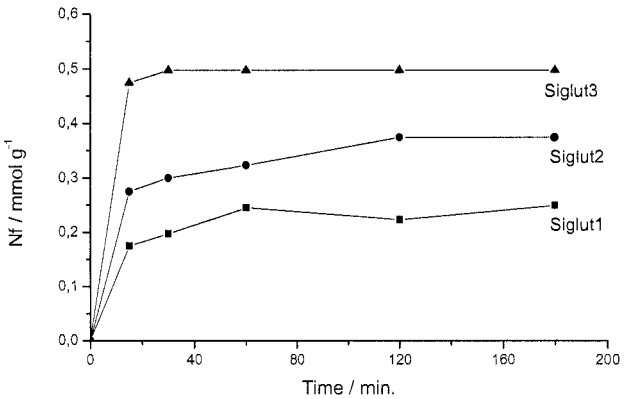


Figure 3. Batch isotherm of adsorption capacity of 0.10 mol dm^{-3} of copper solution at 298 ± 1 K for various intervals of time for Siglut1 (■), Siglut2 (●), Siglut3 (▲) hybrids.

The capacity of immobilization of enzyme was followed through the amount of four different enzymes supported on the hybrids, which procedure consisted in measuring the immobilized fraction after 2 h of contact with buffer solutions, at 298 ± 1 K, and the collected data, in milligram of enzymes per gram of hybrid, are shown in Figure 4.

The amount of immobilized catalase and urease increased significantly from the Siglut1 to the Siglut2 hybrids. However, an opposite behavior was observed for glucose oxidase and invertase, whose values decreased from Siglut1 to Siglut2. On the other hand, the capacity of enzyme immobilization on the Siglut3 hybrid was not possible to be determined due to a probable degradation of this hybrid during the experimental procedure. Enzyme- glutaraldehyde polymer reactions previously studied [22] concluded that the interaction is mediated by hydrophobic as well as electrostatic interactions, between charged enzyme surfaces and the free amino groups disposed on the polymers. Another feature to be considered is the substantial increase in enzymes bonding to polymers modified with glutaraldehyde. Based on the participation of glutaraldehyde in bridging the inorganic parts of the compounds formed, it was observed an increase in the capacity of enzymes immobilization on the hybrids, whose variation is in agreement with the chain size of the organosilane used on the synthetic hybrids. Therefore, the amount of immobilized enzymes increased from Siglut1 to Siglut2 hybrids for catalase, varying from 7.93 to 24.22 mg g^{-1} while for urease, the values 5.30 to 7.91 mg g^{-1} were obtained, but the amount of immobilized enzymes decreases from Siglut1 to Siglut2 for glucose oxidase, varying from 3.02 to 0.89 mg g^{-1}, and invertase decreased from 3.51 to 1.04 mg g^{-1}.

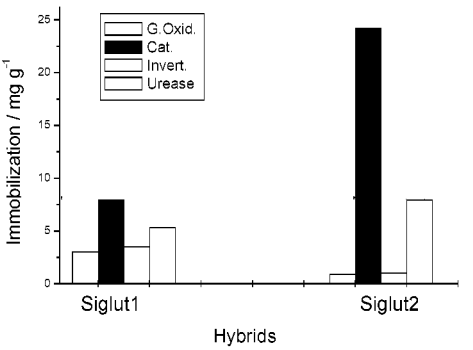


Figure 4. Batch isotherm of immobilization of the enzymes catalase, glucose oxidase, invertase and urease on Siglut1 and Siglut2 hybrids, measured after 2 h of contact with aqueous buffered solution, at 298 ± 1 K.

Conclusion

The synthetic organosilane-glutaraldehyde equimolar hybrids formed from crosslinking and sol-gel processes stages gave solids with pale yellow to yellow colors, manifested by a strongly exothermic reaction. The copper adsorption capacity from aqueous solution increased in agreement with the increase of the organic chain length of the silylant agent used in the synthetic route. The amount of immobilized enzymes increases from Siglut1 to Siglut2 when catalase and urease were employed, with an invertion in capacity for glucose oxidase and invertase. However, Siglut3 showed an odd behavior that could be related to its degradation during the synthetic procedure.

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References

1. Arakaki LNH, Airoldi C. (1999) Quim Nova 22:246
2. Price PM, Clark JM, Macquarrie DJ (2000) J Chem Soc, Dalton Trans 101
4. Mark JE (1996) Heterog Chem Rev 3:307
5. Curriu JPR, Leclercq D (1996) Angew Chem Int Ed 35:1420
6. Cestari AR, Airoldi C (1997) Langmuir 13:2681
7. Giacomini C, Villarino A, Franco-Fraguas L, Batista-Viera F (1998) J Mol Catal B 4:313.
8. Monteiro Junior OAC, Airoldi C (1999) J Colloid Interface Sci 212:212
9. Wuff G (1995) Angew Chem Int Ed Engl 34:1812
10. Rogalski J, Szczodrak J, Pleszczynska M, Fiedurek J (1997) J Mol Catal B 3:271
11. Valkenberg MH, Holderich WF (2002) Catal Rev 44:321
12. Fishman A, Levy I, Cogan U, Shoseyov O (2002) J Mol Catal B 18:121
13 Han YJ, Watson JT, Stucky GD, Buttler (2002) J Mol Catal B 17:1
14. Da Fonseca MG, Barone JS, Airoldi C (2000) Clays Clay Miner 48:638
15. Lowry OH, Rosebrough NJ, Frarr AF, Randall RJ (1951) J Biol Chem 193:265
16. Lima CBA, Airoldi C (2002) Solid State Sci 4:1321
17. Airoldi C, Monteiro Junior ACJ (2000) Appl Polym Sci 77:797
18. Monteiro Junior OAC, Airoldi C (1999) Int J Biol Macromol 26:119
19. Prado AGS, Airoldi C (2002) Green Chem 4:288
20. Ishii H, Minegishi M, Lavitpichayawong B, Mitani T (1995) Int J Biol Macromol 17:21
21. Inoue K, Yoshizuka K, Ohto K (1999) AnalChim Acta 388:209
22. Agarwal R, Gupta MN (1995) Anal Chim Acta 313:25