Hystidine-Salicylaldehyde Schiff Base Cu(II) Complexes Immobilized on Mesoporous Materials as Potentially Biomimetic Oxidation Catalyst

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New potentially oxidation catalysts were synthesized by immobilization of Cu(II) biomimetic complex onto amino-functionalized mesoporous SBA-15 silica supports. The copper complex with Schiff bases derived from hystidine and salicylaldehyde, was tethered onto the pores of a NH_2-functionalized silica. Characterization by XRD analysis, N_2 adsorption-desorption, SEM microscopy, IR and UV-Vis spectroscopy TGA-DTA and elemental analysis confirm the structural integrity of the mesoporous hosts and successful anchoring of the metal complex over the tested support. Catalytic activities of the host free and immobilized complex were tested by oxidation with air of 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid. The activity of the supported metals complex was compared with that of Trametes versicolor laccase, immobilized by the same support.

Keywords: SBA-15, Cu (II) complexes, Schiff bases, laccase, biomimetic catalysts

There has been considerable interest in recent years in the development of models which mimic chemical aspects of the metalloenzymes occur in nature. Copper containing enzymes are utilized for electron transfer (galactose oxidase, azurins, and laccases), for oxidation and oxygenation reactions (polyphenol oxidase, ascorbate oxidase, and hemocyanin) and for superoxide dismutation (Cu, Zn-superoxide dismutase).The role played by copper ions in the active sites of these enzymes has stimulated efforts to design and characterize copper complexes as models for a better understanding of biological systems [1-3]. Copper Schiff base complexes are among the most versatile catalysts known for oxygenation reactions. However, these homogeneous complexes suffer from poor catalyst recovery and difficult product separation. Thus, over the past decade much effort has been done for the heterogenization of the known efficient homogeneous complexes and covalent anchoring of the Schiff base complexes onto a functionalized siliceous mesoporous material with large pore diameters seems to be promising. SBA-15 appears to be one of the best candidates for catalytic support due to its regular pore size, a large surface area, a large number of surface silanol groups, and a high chemical and thermal stability [4]. Functionalized alkoxysilanes, such as 3-aminopropyltriethoxysilane, allow the subsequent bounding of the catalytic active species. Recently, Cu(II) tetrahydro-salen complexes have been successfully immobilized through aminosilane linkers and were applied for catalytic reactions [5]. Therefore, in our investigation, a amino acid Schiff base complex was prepared and then immobilized onto SBA-15 containing regular mesoporous pores by flexible aminopropyl linkers. The obtained immobilized catalysts were used for the oxidation reaction of 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid with air. The biomimetic behaviour of the immobilized copper complexes was evidenced by comparing their oxidative catalytic activity with that of the laccase immobilized in the same supports.

Experimental part

Preparation of functionalized SBA-15 silica

SBA-15 material was synthesized as described in literature [6]. Thus, 1.5 g of amphiphilic triblock copolymer (Pluronic P123, EO_{20}-PO_{70}-EO_{20}, average molecular weight 5800, BASF) was dispersed in 15 g of H_2O and 45 g of 2 M HCl with stirring at 313 K for 4 h. 3.15 g of tetraethylorthosilicate (TEOS, 98% Aldrich) was added and the obtained homogeneous solution was stirred at 40°C for 24 h. The resulting gel was then hydrothermally treated in a Teflon-lined autoclave at 100°C for 2 days. The solid was centrifuged, filtered, washed with deionized water, and dried in air at room temperature. The as-synthesized SBA-15 was calcined at 550°C during 8 h under air flow.

The organic-inorganic hybrid material was obtained by a post-grafting procedure with 3-aminopropyltriethoxysilane (APTES) according to a previously described procedure [7]. 1g of SBA-15 silica, freshly activated overnight at 130°C under vacuum, and 1 mL of APTES (99% Aldrich) were added to 50 mL of dry toluene. After stirring the solution at reflux of toluene for 2 h, the ethanol released was distilled off and the mixture was kept under reflux for 90 min. The NH_2-functionalized mesoporous silica (referred as NH_2-SBA-15) was filtered and washed with toluene, ethylic alcohol and then diethyl ether. It was then submitted to a continuous extraction run overnight in a Soxhlet apparatus using diethyl ether/dichloromethane (v/v, 1/1) at 100°C and dried overnight at 130°C.

Synthesis of hystidine-salicylaldehyde Schiff base Cu(II) complex and its heterogenization

The Cu(II) complex was synthesized as described in literature [8]. L-hystidine (10 mmols) were added into a methanolic solution (50 mL) of NaOH (20 mmols). Salicylaldehyde (10 mmols) dissolved in 50 mL methanol was added thereafter under magnetic stirring onto the amino acid solution. After 2 min. copper acetate (5 mmols)

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was added and the mixture was kept under continuous stirring for 3h at room temperature. The volume was thereafter reduced to 1/4 of the initial value (20 mL) and the solid was filtered. A mixture of methanol-ethanol (2:1) was used for complex recristalisation. The obtained green precipitate was the desired homogeneous complex hystidine-salicylaldehyde Schiff base copper (II) complex and it was denoted Cu(Sal-His). Elemental analysis (%), Cu(Sal-His), $C_{13}H_{17}N_3O_5Cu$, measured value(calculated value): C, 43.41(43.45); H, 3.60(3.62); N, 11.72(11.70); Cu, 17.70(17.69); N/Cr molar ratio = 3.01:1.00.

Since the copper center in the homogeneous complex was five-coordinate, the immobilization of the homogeneous complex onto SBA-15 was readily achieved by the copper coordination to the amino nitrogen of NH_2-SBA-15 by simple addition [9]. Typically, the Cu(II) complex (0.6 g) in 50 mL dry toluene was added to 1g of freshly activated amine-functionalized SBA-15 silica support suspended in 50 mL dry toluene. The mixture was vigorously stirred under reflux for 10 h. After cooling, the suspension was filtered and the solid was washed overnight in a Soxhlet extractor using equivalent alcohol and acetonitrile as solvent to remove the homogeneous complexes adsorbed on the surface of the support, and then the solid was dried in air at 80°C for 10 h. The obtained green sample was the expected immobilized complex hystidine–salicylaldehyde Schiff base copper (II) complex and it was denoted Cu(Sal-His)-SBA-15.

Enzyme immobilization

Laccase from *T. versicolor* (Sigma) with an activity of 34 I.U· mg^{-1} protein was used without further purification. Immobilization of laccase was carried out by physical adsorption. In a typical procedure, 1 g support (NH_2-SBA-15) was mixed with 10 mL of 0.1 M phosphate buffer (pH 7.0) in a centrifuge tube. Thereafter, 50 mg laccase in 3 mL phosphate buffer were added to the mixture and homogenized under magnetic stirring at 5°C. In this suspension acetone (30 mL) was added drop wise and the stirring was continued for another 30 minutes. The solid was isolated by centrifugation and washed several times with buffer until no laccase activity was detected in the washing.

Characterization

Small-angle XRD data were acquired on a Bruker AXS D8 diffractometer by using Cu Kα radiation and Ni filter. N_2 adsorption–desorption isotherms were measured at 77 K with a Micromeritics ASAP 2010 instrument. The sample was previously degassed under vacuum at 323 K for 12 h. Specific surface area was calculated by the BET method and the mesopore volume was determined from the isotherm at the end of capillary condensation. The pores size distribution was obtained from the desorption branch using the BJH method and the Harkins-Jura standard isotherm. FTIR spectra of all samples were performed in KBr pellets using a Bruker Alpha spectrometer. The UV-Vis diffuse reflectance spectra were recorded using a Thermo Scientific (Evolution 600) spectrometer. Atomic adsorption spectroscopy (FAAS) measurements were performed on a GBS Avanta spectrometer equipped with multi-element hollow cathode lamps and air-acetylene burner. C, H, and N contents were evaluated by combustion on a Fisons EA1108 elemental analysis apparatus. Thermogravimetric analysis was carried out in a Netzsch TG 209C thermobalance.

Catalytic activity measurements

Catalytic activities of the host free and immobilized complex were tested by oxidation with air of 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) in the same maner as for the free and immobilized laccase. The results were evaluated from the absorption band of ABTS oxidation product at 424 nm (ε = 36,000 L mol^{-1} cm^{-1}) and 30°C. The *p*H value was kept at 4.5 with acetate buffer and the solution was saturated in oxygen by air bubbling before the starting of the catalytic test. In such conditions, an enzyme solution of one unit/mL (U.I./mL) was required to oxidize 1μmol ABTS per minute. For the determination of immobilized laccase activity, 10 mg of active solids were added to 50 mL of 1mM ABTS in 0.1 M acetate buffer (*p*H 4.5), which was allowed to circulate through the spectrophotometric cell termostatated at 30°C. The enzymatic activity was expressed in U.I./mg of immobilized protein or as relative activity:

$$E.A.\% = \frac{U^{solid}/mg}{U^{solution}/mg} \times 100$$

For the immobilized complex the amounts of active solids were calculated in order to maintain the same amount of Cu(II) as in aqueous solution of complex. During the catalytic reaction, the absorbance was measured for 60 min and the initial rate of ABTS oxidation was obtained from the plots of ABTS oxidation product concentrations versus time.

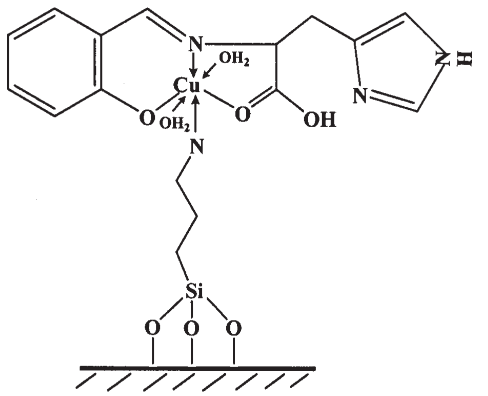
Results and discussions

Spectroscopic characterization of free and immobilized complexes

The FTIR spectra for the free and immobilized complex (fig. 1) have been registered in the 4000 - 400 cm^{-1} range. The important bands from the spectroscopic characterization were assigned and the band positions are listed in table 1. The characteristic bands indicating the successful preparation of the amino acid Schiff base complex, namely ν(C=N), $\nu_{as}(COO^-)$, $\nu_s(COO^-)$, were all present in the FT-IR spectra of the homogeneous complex, and the band positions agree well with published data [9-11]. Furthermore, the complex spectra contain new bands in the 800-600 cm^{-1} region that could be attributed to the ν(Cu-O) (phenolic oxygen), 701-500 cm^{-1}, that could be attributed to ν(Cu-O)(carboxylic oxygen) and 500 - 600 cm^{-1} respectively, for the ν(Cu-N) valence vibration [12].

In the FT-IR spectra of the immobilized complexes, apart from the bands in the overlapping regions of the silica backbone, the other bands of the homogeneous complexes were all clearly present (fig. 1a and table 1) indicating the successful immobilization of the homogeneous complexes onto SBA-15. However, compared with their corresponding homogeneous complexes the intensity of the characteristic bands of the immobilized complexes decreased. This is mainly attributed to the low loading of copper complex.

The UV-Vis spectra of the homogeneous complex displayed two typical peaks at 387 nm and 600 nm respectively; the former could be assigned to metal-ligand transition bands and the latter is associated with a *d-d* transition [13] (fig. 1b and table 1). This also proves the successful preparation of the homogeneous complex. The UV-Vis spectrum of the immobilized complex was similar to the spectra of its corresponding homogeneous analogue (fig. 1 and table 1), further confirming that the immobilized complex depicted in scheme 1 was successfully prepared.



Scheme 1. Structural representation of the heterogenized cooper complex

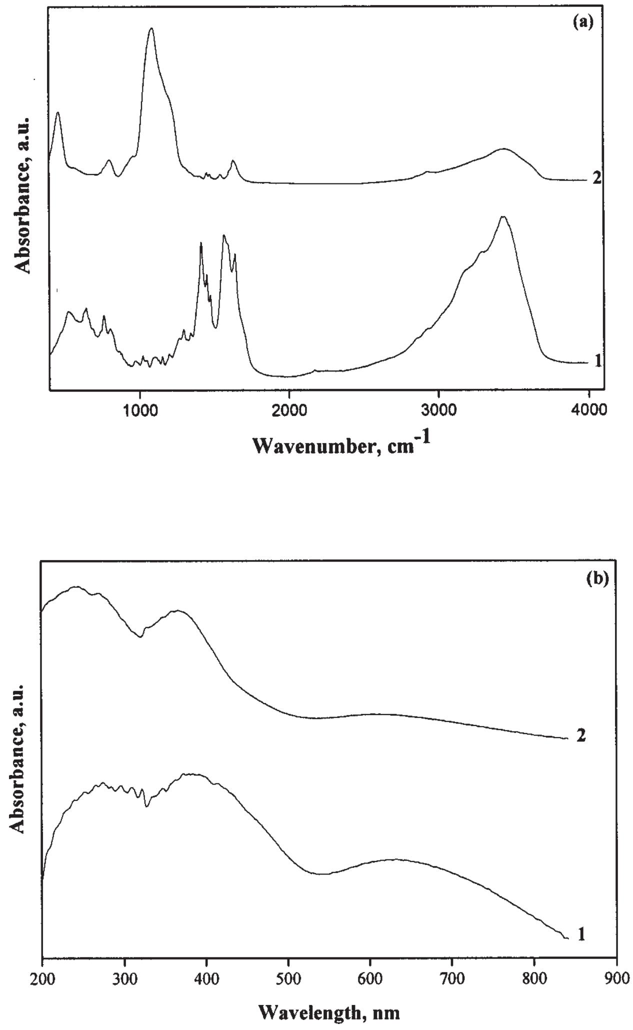


Fig. 1. FT-IR (a) and UV-Vis (b) spectra of the copper complex. (1) Cu(Sal-His); (2) Cu(Sal-His)-SBA-15

Phase structures of the catalysts

The powder XRD patterns for SBA-15, SBA-15-NH_2 and Cu(Sal-His)-SBA-15 are depicted in figure .2. The SBA-15 material exhibited a strong (100) reflection peak (at $2\theta = 0.90^0$) and two smaller diffraction peaks (110 and 200) at 1.52° and 1.67°, respectively, that can be indexed as a hexagonal lattice with *d*- spacing values of 98.12, 57.91 and 53.12 Å, respectively. The unit cell parameter, a_o, was of 113 Å.

No significant changes upon amine immobilization were observed, except for the expected decrease in XRD peak intensity, providing the evidence that functionalization occurred mainly inside the mesopore channels.

For the hybrid materials, the relative intensity of the proeminent diffraction peak (100) decreased after the introduction of the Schiff base-copper complex. The intensity reduction may be mainly due to contrast matching between the silica framework and the organometallic moieties located inside the SBA-15 channels.

The N_2 adsorption/desorption isotherms of SBA-15 and the hybrid materials are shown in figure 3. The mesoporous

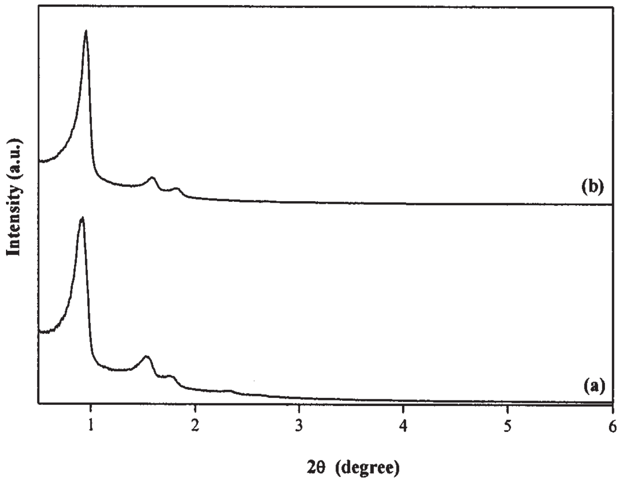


Fig. 2. XRD patterns of (a) SBA-15 and (b) Cu(Sal-His)-SBA-15

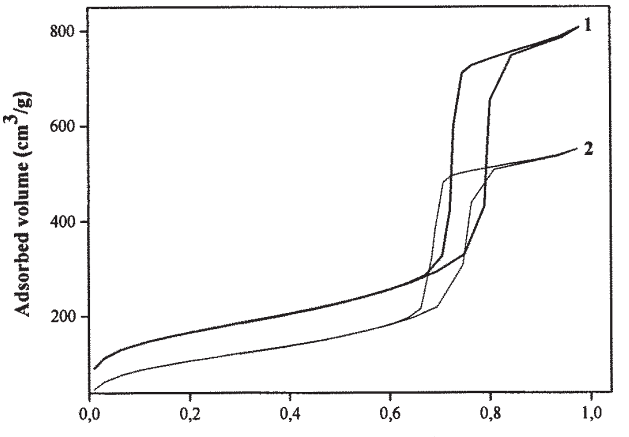


Fig. 3. N_2 adsorption-desorption isotherms of SBA-15 (1) and Cu(Sal-His)-SBA-15 (2) samples

Table 1
DATA FROM FT-IR AND UV-VIS SPECTRA OF THE COPPER COMPLEXES

Sample	FT-IR (cm^{-1})							UV-VIS (nm)	
	υ(C=N)	υ_{as}(COO$^-$)	υ_s(COO$^-$)	ν_{Ph-O}	ν_{Cr-O}^a	ν_{Cr-O}^b	ν_{Cr-N}	*p-π**	*d-d*
Cu(Sal-His	1631	1561	1403	1338	760	643	526	387	600
Cu(Sal-His)-SBA-15	1629	1541	1400	1311	755	623	529	387	600

[a]Phenolic oxygen; [b]Carboxylic oxygen.

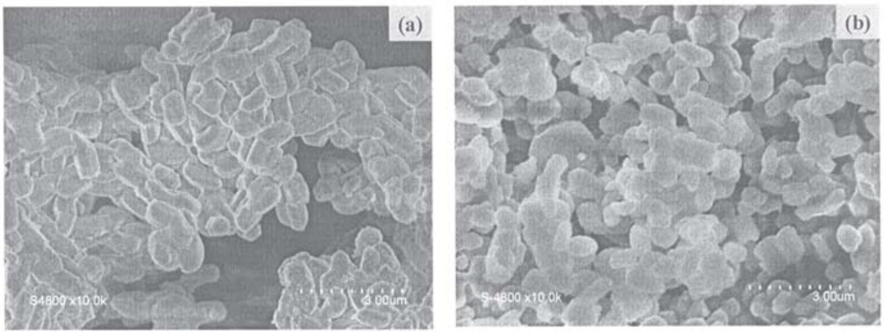


Fig. 4. SEM images of SBA-15 (a) and Cu(Sal-His)-SBA-15 (b)

Table 2
TEXTURAL PROPERTIES OF CALCINED AND MODIFIED SBA-15[a]

Sample	A_{BET} ($m^2 g^{-1}$)	V_p (mL g^{-1})	d_p (nm)
SBA-15	600	1.1	7.8
Cu(Sal-His)-SBA-15	398	0.8	6.6

[a]A_{BET} , specific surface area; V_p, pore volume;d_p, pore diameter.

Table 3
ELEMENTAL AND TGA ANALYSIS RESULTS

Sample	Elemental analysis					Complex amount [a]		Immobilization yield%
	Copper amount		C%	N%	H%	mg g^{-1}	mmol g^{-1}	
	mg g^{-1}	mmol g^{-1}						
Cu(Sal-His)-SBA-15	100.97	1.59	2.40	0.65	0.2	551.45	1.54	91.91

[a]Determined by TGA.

silica materials exhibited adsorption–desorption isotherms with a H1 hysteresis loop in the partial pressure range from 0.50 to 0.80, characteristic of materials with 7–8 nm pore diameter. Obviously, the hybrid material maintained the characteristics of type IV isotherms. This result reveals that the uniform mesoporous nature of the material is preserved even though the grafting has occurred. The main textural properties of solids are listed in table 2. However all the textural parameters were diminished upon the copper complex immobilization. These results confirm that the grafted species are located inside the mesopores and not only on the outer surface.

A SEM image of Cu(Sal-His)-SBA-15 showed many rope-like domain with a relatively uniform length of $3\mu m$(fig. 4b). These domains were aggregated to a wheat-like macrostructure similar to earlier reported morphologies of parent SBA-15 (fig.4a), indicating that the immobilization procedure does not influence the morphology of the pure siliceous SBA-15.

TGA studies and immobilization yields

Based on elemental and thermogravimetric analysis, the amount of metals, complexes and immobilization yield were determined (table 3). The copper contents of all samples were determined by FAA spectroscopy after their dissolution in 10% HF.

Due to the mesoporous open stucture of the SBA-15 silica and to the presence of the surface amino group that could coordinate to the copper ions, the immobilization yield of salicylaldehyde-hystidine Schiff base Cu(II) complex was very high.

The TG/DTA profiles of the Cu(Sal-His)-SBA-15 indicate that immobilization was successful on the tested support material. The weight loss at temperatures below 200^{0}C would be attributed to the loss of adsorbed water. The thermal decomposition of the immobilized complex occurred between 270^{0}C and 580^{0}C (fig. 4).

Trametes versicolor laccase is a globular protein having 5x5x7 nm dimensions and a molecular weight of 60 kDa. These dimensions allow the enzyme accomodation into micro- and mezopores with diameters higer than 10 nm. Trametes versicolor laccase was immobilized by adsorption into an mesopoporous silica support. The amount of immobilized enzyme determined as organic mass loss from TG-DTA curves was 81.9 mg g^{-1} (67.33% immobilization yield). The high yield obtained for the laccase immobilization into the amino-functionalized SBA-15 silica is probably due to the stronger interactions between the enzyme functional groups (-NH_2, -COOH, -OH, -SH) and that of the support surface.

Catalytic activity

We mention that similar complexes but with Cr(III) cation and alanine, glycine or phenylalanine aminoacids were immobilized into SBA-15 or MCM-41 supports and tested in the liquid phase styrene or cyclohexene epoxidation reaction [9,14]. There are not data about the biomimetic laccase activity tests for Cu(Sal-His) complex free or immobilized.

Laccases (p-diphenol: dioxygen oxidoreductase, E.C. 1.10.3.2) are multi-copper oxidase and reduce oxygen directly to water in four-electron transfer step without intermediate formation of soluble hydrogen peroxide in expense of one-electron oxidation of a variety of substrates, e.g., phenolic compounds [15]. Trametes versicolor laccase was immobilized by adsorption into amino-functionalized SBA-15 mesoporous silica supports in order to obtain an efficient catalyst for the ABTS substrate oxidation. The

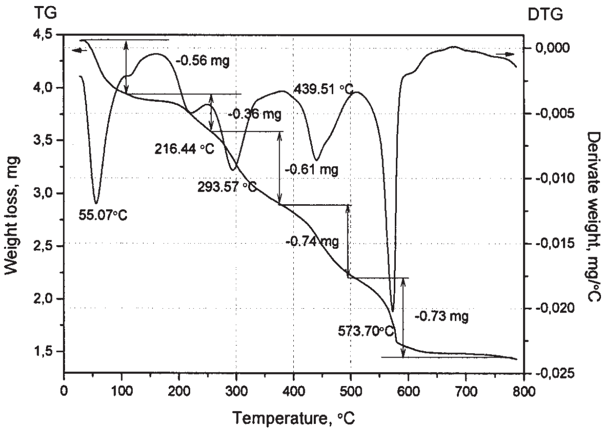


Fig. 5. TG/DTG profiles for Cu(Sal-His)-SBA-15 sample

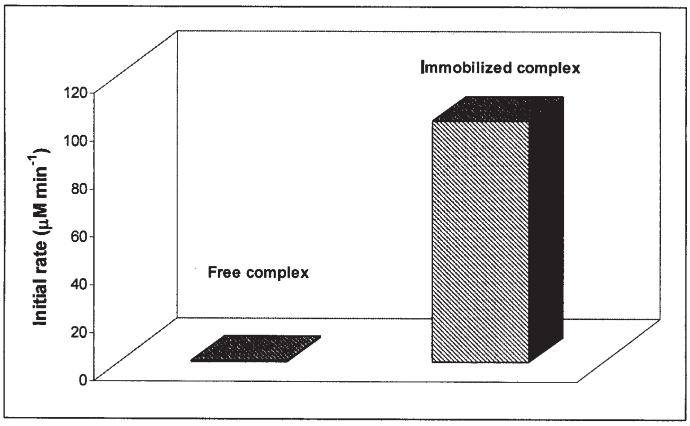


Fig. 6. Initial rate values of host-free and immobilized copper (II) complex in the ABTS oxidation reaction

oxidative catalytic activity of this one was compared with those of the immobilised copper complex.

ABTS oxidation measurements revealed the catalytic activities of the free or immobilized complex. The initial rate values (calculated from the concentration of ABTS oxidation product versus time graph) are given in figure 5.

The calculated initial rate value o f the host-free copper complex was $0.50\mu M\times min^{-1}$. The activity of the complex significantly changed upon immobilization. The initial rate of the ABTS oxidation was higher for Cu(Sal-His)-SBA-15 than for the host-free complex. Thus, for Cu(Sal-His) immobilized into SBA-15-NH_2 supports, activity was 100.95 $\mu M\ .\ min^{-1}$, 200 time higher than the oxidative activity of the free complex. This is probably due to additional interactions of the substrate with the support matrix. Furthermore, this result proves the biomimetic oxidative activity of the tested complex. We mention that this one could present biological activity too as it was the case for other complexes with Schiff base derived from aminoacids previously synthesized [16].

The activity of free laccase towards ABTS oxidation with air was firstly determined (33.3 U/mg). The ezimatic activity of the immobilized laccase was 27.75 U.I/mg, with a relative activity of 87.1%, respectively. The obtained values for the enzymatic activity and the relative activity of the immobilized laccase suggest that the immobilized laccase posses a much higher oxidative catalytic activity (about 10^3 times) than the biomimetic catalysts. Unlike complexes, a decrease of the enzymatic activity was evidenced upon enzyme immobilization. This behavior can be attributed to the more difficult diffusion of the substrate to the active sites of the enzymes due to their tertiary structure.

Conclusions

New biomimetic catalysts based on hystidine-salicylaldehyde Schiff base Cu(II) complex and of *Trametes versicolor* laccase immobilized on NH_2- functionalized SBA-15 mesoporous silica supports have been synthesized. The successful anchoring of the catalysts over the supports and the structural integrity of the host materials were confirmed by various characterization techniques. The oxidation activities of complexes after immobilization have been proved to be higher than in homogeneous phase.

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References

1.SOLOMON, E.I., CHEN, P., METZ, M., LEE, S.-K., PALMER, A.E., Angew. Chem., Int. Ed. Engl., **40**, 2001, p. 4570–4590.
2.ABOLMAALI, B., TAYLOR, H.V., WESER, U., Struct. Bonding, **9**, 1998, p. 91–190.
3.KAIM, W., RALL, J., Angew. Chem., **108**, 1996, p. 47–64.
4.TADA, M., COQUET, R., YOSHIDA, J., KINOSHITA, M., IWASAWA, Y., Angew. Chem. Int. Ed., **46**, 2007, p. 7220.
5.YANG, Y., ZHANG, Y., HAO, S., KAN, Q., Chem. Eng. J., **171**, 2011, p. 1356.
6.ZHAO, D., FENG, J., HUO, Q., MELOSH, N., FREDRICKSON, G.H., CHMELKA, B.F., STUCKY, G.D., Science, **279**, 1998, p. 548.7. MARTIN,T., GALARNEAU, A., BRUNEL, D., IZARD, V., HULEA, V., BLANC, A.C., ABRAMSON, S., RENZO, F. DI, FAJULA, F., Stud. Surf. Sci. Catal., **135**, 2001, p. 4621.
8.SAKYIAN, I., GUNDUZ, N., GUNDUZ, T., Synth React Inorg Met-Org Chem, **31** , no. 7, 2001, p. 1175-1187.
9.WANG, X., WU, G., WEI, W., SUN, Y., Catal. Lett., **136**, 2010, p. 96.
10.PERCY, G. C., J Inorg Nucl Chem, **37**, 1975, p. 2071.
11.HAN, J. P., ZHAO, J. Q., ZHANG, Y. C., LU, J. G., J Hebei Univ Technol, **33**, no. 4, 2004, p. 80.
12.DIAZ, I., MARQUEZ-ALVAREZ, C., MOHINO F., PEREZ-PARIENTE, J. , SASTRE, E. J Catal, **193**, 2000, p. 283.
13.REN, T., YAN, L., ZHANG, H. P., SUO, J. SH., J Mol Catal(China), **19**, 2003, p.310
14.WANG, X., WU, G., WANG, F., WEI, W., SUN, Y., Chinese Journal of Catalysis, **32** no.12, 2011, p. 1812.
15.TIMUR, S., PAZARLIOGLU, N., PILLOTON, R., TELEFONCU, A., Sens. Actuators, **B 97**, 2004, p. 132.
16.CIOLAN, F., PATRON, L., MURESEANU, M., ROTARU, P., GEORGESCU, I., Rev. Chim. (Bucharest), **63**, no. 1, 2012, p. 34-39.

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