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Immobilization of glucose oxidase enzyme (GOD) in large pore ordered mesoporous cage-like FDU-1 silica

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ABSTRACT

Large pore ordered mesoporous silica FDU-1 with three-dimensional (3D) face-centered cubic, Fm3m arrangement of mesopores, was synthesized under strong acid media using B-50-6600 poly(ethylene oxide)–poly(butylene oxide)–poly(ethylene oxide) triblock copolymer ($EO_{39}BO_{47}EO_{39}$), tetraethyl orthosilicate (TEOS) and trimethyl-benzene (TMB). Large pore FDU-1 silica was obtained by using the following gel composition 1TEOS:0.00735B50-6600:0.00735TMB:6HCl:155H_2O. The pristine material exhibited a BET specific surface area of 684 $m^2\,g^{-1}$, total pore volume of 0.89 $cm^3\,g^{-1}$, external surface area of 49 $m^2\,g^{-1}$ and microporous volume of 0.09 $cm^3\,g^{-1}$. The enzyme activity was determined by the Flow Injection Analysis-Chemiluminescence (FIA-CL) method. For GOD immobilized on the FDU-1 silica, GOD supernatant and GOD solution, the FIA-CL results were 9.0, 18.6 and 34.0 U, respectively. The value obtained for the activity of the GOD solution with FIA-CL method is in agreement with the 35 U, obtained by spectrophotometry.

1. Introduction

Since the discovery of ordered mesoporous silica (OMS) by the researchers of the Mobil Company in 1992 [1,2] significant progress has been made in the synthesis, characterization, and application of ordered mesoporous materials, which can be prepared using either ionic or nonionic surfactant templates. The latter has many advantages in comparison to ionic surfactants, such as biodegradability, commercial availability, low cost and low toxicity [3,4]. Yu et al. [5,6] synthesized an interesting OMS, the FDU-1, with a 3D cubic structure having large cage-like mesopores. In this case, a poly(ethylene oxide)–poly(butylene oxide)–poly(ethylene oxide) triblock copolymer (PEO–PBO–PEO) with 39 and 47 PEO and PBO blocks, respectively, was used as a structure directing agent. The polymer-templated cage-like OMSs have attracted a lot of worldwide attention because of their properties [7–11], as well as their potential applications in catalysis [12], adsorption [13], immobilization of biomolecules and enzymes [14] and sensing devices [15]. In particular, the fixation of biologically active species onto inorganic materials combines the high selectivity of enzymatic reactions with the chemical and mechanical properties of the support, which acts as a protective barrier, improving the time activity of the enzymes. The cage like structure of FDU-1 is suitable for the encapsulation of organic molecules, since it posses a pore entrance of 2–4 nm and a cage diameter of 10–15 nm, typically. Methods of physical immobilization of enzymes include covalent attachment or adsorption to a support surface, semi-permeable membrane or sol–gel entrapment, and microencapsulation, for which comprehensive reviews are available [16,17]. The immobilization technique should allow the enzyme to maintain its catalytic activity while diminishing other processes that are detrimental to the enzyme, such as autolysis. The support should allow the immobilization or encapsulation of large quantities of enzyme with good enzyme–substrate contact, as well as provide a robust physical and chemical environment [18,19].

In this scenario the aim of this article was to demonstrate the ability of large pore ordered FDU-1 silica to immobilize GOD molecules and to describe a method to measure the catalytic activity of an OMS, like the particular case of FDU-1 with GOD, since the material is opaque to visible light and cannot be analyzed by absorption methods.

In this work, the glucose oxidase (GOD) immobilized in large pore FDU-1 silica was investigated. Recent works report the immobilization of GOD in SBA-15 silica and its application as a modified electrode in voltammetric techniques [20] and biocatalyst [21], GOD is a dimmeric protein with a molar mass of 160 kDa, which catalyses the oxidation of β-D-glucose by oxygen to D-glucono-1,5-

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lactone and hydrogen peroxide. GOD has a number of industrial applications due to its ability to remove glucose and O_2 or production of gluconic acid and H_2O_2. GOD is used for desugaring of eggs in egg-solids, production of reduced alcohol wines, removal of oxygen and browning control to increase storage time of fruit juices, purees, mayonnaise and other tinned foods. GOD is also used as an alternative route to produce H_2O_2 for textile bleaching or milk pasteurization. Gluconic acid and its derivatives have been produced by using GOD in large quantities for pharmaceutical and industrial applications [22]. Immobilization of GOD can offer several advantages including its reuse, ease in application of both batch and continuous systems, possibility of better control of reactions, ease removal from the reaction medium and improved stability [19].

For the first time, this article reports the immobilization of GOD on the large pore FDU-1 silica and proves the feasibility of enzymatic activity measurements in the immobilized enzyme by means of a FIA-CL procedure.

The relevant characteristic of the immobilized enzyme is its activity, which can be determined by spectrophotometry [23] and by chemiluminescence (CL) [24]. Both procedures determine the hydrogen peroxide produced in the reaction between D-glucose and O_2. Spectrophotometric determination is based on the use of o-dianisidine (colorless), which reacts with peroxide in presence peroxidase to produce an o-dianisidine oxidized form (brown). This compound reacts with sulfuric acid to form a more stable product measured at 540 nm, whose absorbance is proportional to the glucose concentration. Because of the kinetic nature of this determination, batch experiments need strict time control to achieve reliable results, a difficulty that can be circumvented by FIA procedures. Chemiluminescence measurements are made in a FIA system employing the oxidation of luminol by the hydrogen peroxide produced in the reaction between O_2 and glucose catalyzed by GOD. The reaction between luminol and H_2O_2 occurs in alkaline medium and is catalyzed by potassium hexacyanoferrate (III). In CL this radiation is emitted in the course of the chemical reaction, when an excited atom or molecule decay to the ground state. The CL systems are highly efficient and based on synthetic molecules such as luminol [19–26]. Methods based on CL have the advantages of high sensitivity and use of simple instrumentation. The main drawback of CL detection is the need for an appropriate and strict control of the reaction time, a task that can be achieved by FIA.

2. Materials and methods

2.1. Synthesis

The synthesis of the FDU-1 silica was carried out using the same synthesis gel composition as reported Yu et al. [4–6] except for adittion of trimethyl-benzene (TMB), a micelle expander, 1TEOS:0.00735 B50-6600:0.00735 TMB:6 HCl:155 H_2O, where TEOS stands for tetraethyl orthosilicate from Aldrich Chemical Company and B50-6600 is a poly(ethylene oxide)–poly(butylene oxide)–poly(ethylene oxide) triblock copolymer, $EO_{39}BO_{47}EO_{39}$ from Dow Chemicals. In the synthesis procedure 2 g of B50-6600 copolymer and 35.3 mg (41 μL) of TMB was dissolved in 120 g of a 2 M HCl aqueous solution and stirred at 25 °C until a homogeneous mixture is obtained. Subsequently, 8.32 g (8.92 mL) of TEOS was added and the resulting mixture was stirred vigorously in an open beaker for 24 h at 25 °C. The precipitation is usually observed 30–40 min after the addition of TEOS. The mixture is submitted to a hydrothermal treatment by transferring it to a Teflon-lined autoclave kept under static conditions at 100 °C during 24 h. Finally, the precipitate was filtered out, washed with distilled water and dried at 25 °C and calcinated under nitrogen, which was afterwards switched to air, at 540 °C.

2.2. Glucose oxidase immobilization

One hundred mg of FDU-1 silica was suspended in 0.1 mol L^{-1} acetate buffer pH = 4.5 in a reactor coupled to a refrigeration system regulated at 4 °C. After the suspension reached the thermal equilibrium, about 100 U mL^{-1} GOD (nominal value) were added and the system, which was kept at 4 °C under magnetic stirring for 12 h. At the end of this period the suspension was filtered in Millipore system (0.45 μm). The supernatant solution was collected and stored under refrigeration [27].

2.3. Methods

TG/DTG curves were obtained between 25 and 900 °C with 5 mg of samples in a TGA 50 Thermobalance from Shimadzu using platinum crucibles, under dynamic air atmosphere (50 mL min^{-1}) and heating rate of 10 °C min^{-1}.

The SAXS experiments were carried out in a rotating anode X-ray generator (Rigaku), operating at 10 kW, 40 mA. The wavelength of the copper monochromatic X-ray beam was λ = 0.15418 nm. An image plate detector was utilized to record the scattering intensity as a function of the scattering vector, $q = (4\pi \sin\theta)/\lambda$, θ being half the scattering angle. The intensity was recorded during 2 h. The line focus geometry was used and the system was collimated by slits. A vacuum path between the sample and the detector was utilized. The scattering of the sample's holder was subtracted from the total measured intensities. The sample detector distance (~480 mm) was chosen in order to record the scattering for q values ranging from 0.08 nm^{-1} to 3.5 nm^{-1}. The samples were placed inside a quartz tube, 1.5 mm in diameter.

Adsorption isotherms were measured with Micromeritics ASAP 2010 volumetric adsorption analyzer using nitrogen of 99.998% purity. Measurements were performed in the range of relative pressure from 10^{-6} to 0.99 liquid nitrogen on the samples degassed for 2 h, under vacuum of about 10^{-3} Torr, at 110 °C. The degassing temperature (110 °C) was selected to avoid the decomposition of organic molecules and ensure thermodecomposition of physically adsorbed water. The specific surface area was evaluated using BET method [28]. The total pore volume was estimated from the amount adsorbed at the relative pressure of 0.99. The pore size distribution (PSD) was calculated using Kruk, Jaroniec and Sayari (KJS) method [29].

For transmission electron microscopy (TEM), the sample was studied using a LEO 906E. The FDU-1 sample was collected in a tip of a needle and placed inside the hole of capsule Beem #1. Then the capsule was filled with resin stick (Embed-812, Electron Microscopy Sciences) and centrifuged to 14,000 rpm for 2 min in a centrifugal Jouan A14. The polymerization of the resin was conducted in an oven at 60 °C for 4 days. Ultrathin sections were obtained about 60 nm obtained in an Ultramicrotono MT6000 Sorvall, put on a 300 mesh Cu grid and then examined in a TEM to 80 or 100 kV.

2.4. Spectrophotometric determination of GOD activity

A GOD solution was prepared by dissolving 200 μL of GOD stock in 50 mL (~100 U) of 0.1 mol L^{-1} acetate buffer solution, pH = 4.5, at 4 °C, in a glass reactor coupled to a cooled bath. An aliquot of 20 μL of this solution was transferred to a quartz cell of 1.00 cm, containing a solution with the following reagents: 2.50 mL of 0.066 mg mL^{-1} o-dianizidine–HCl^{-1} in 0.1 mol L^{-1} phosphate buffer pH = 7, 0.50 mL of 100 mg mL^{-1} D-glucose and 10 μL of 2 mg mL^{-1} horseradish peroxidase enzyme. The signal of absorbance vs. time was measured

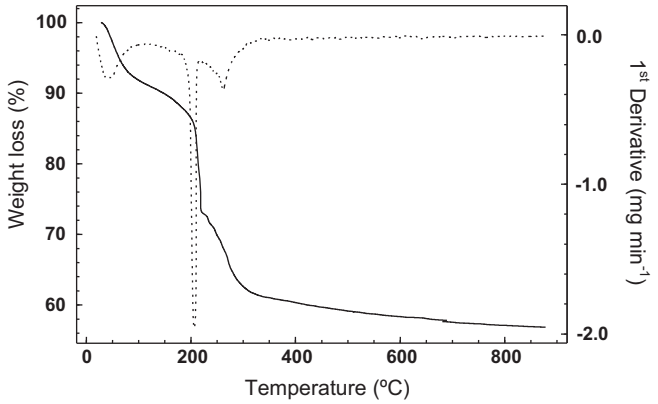


Fig. 1. TG/DTG curves of the FDU-1 silica as-synthesized sample obtained in dynamic air atmosphere and heating rate of 10 °C min^{-1} until 900 °C.

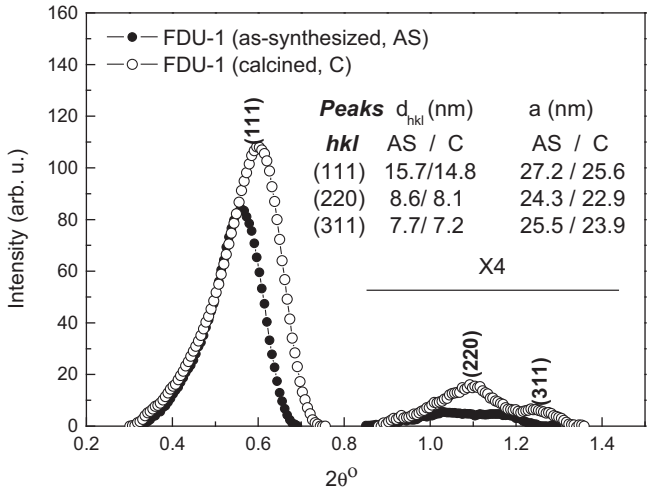


Fig. 2. Small angle X-ray scattering (SAXS) data of the FDU-1 silica: as-synthesized and calcined. For better view of the peaks, a background (BG) removal of the isotropic scattering was performed and less intense data were multiplied by a factor of 4×.

in a spectrophotometer HITACHI model U3000, in the wavelength of 436 nm in 0.1 mol L^{-1} phosphate buffer; pH = 7.0 and 25 °C.

2.5. Determination of the activity immobilized GOD in large pore FDU-1 by FIA-CL

A FIA module was implemented with polyethylene tubes (0.8 mm i.d.), Perspex joint points and a sliding-bar injector for the sampling. A peristaltic pump (Ismatec CPI-4) equipped with Tygon tubes was employed for fluid propulsion. A model 422 Femto spectrophotometer (Femto, São Paulo, Brazil) was employed to measure the emitted radiation. Radiation of the spectrophotometer lamp was blocked by placing an Al shield between the lamp and the detection cell. A flow cell was built with a tube of polyethylene (0.1 mm in thickness and 0.8 mm i.d.) coiled in spiral [24].

The enzymatic reaction was processed in a doubled wall cell through which water was circulated from a thermostatic bath (Tecnal ET 184) regulated at 35.0 ± 0.2 °C. An aliquot of 200 mL of 3.90×10^{-3} mol L^{-1} glucose solution was maintained for 15 min at 35 °C to reach the thermal equilibrium. Then, the FDU-1 silica obtained after the process of GOD immobilization was added to the glucose solution and then the timer was actuated. A tube inserted in the reactor allowed the sampling of aliquots from the reaction mixture at intervals of 1 min. The peroxide contained in the sample oxidizes the luminol in buffered medium of 0.5 mol L^{-1} $KHCO_3/K_2CO_3$ (pH = 10.5) in presence of the catalyst $K_3[Fe(CN)_6]$. The enzymatic activitiy of the GOD stock solution and the activity of the supernatant solution obtained from the filtration of GOD immobilization on FDU-1 silica were also measured.

3. Results and discussions

3.1. Material characterization

The TG/DTG curves of as-synthesized FDU-1 sample are shown in Fig. 1. The curve exhibits three steps of mass loss. The 1st step, Δm = 9.3%, occurs between 25 and 150 °C, T_{peaks} in DTG = 55 °C corresponds to physiosorbed water elimination. The 2nd step of mass loss, Δm = 17.9%, occurs between 150 and 220 °C, T_{peaks} in DTG = 215 °C, corresponding to the first step of volatilization and/or decomposition of templates. The 3rd step of mass loss, Δm = 11.6% occurs between 220 and 330 °C, T_{peaks} in DTG = 270 °C, and can be assigned to the initial process of thermal volatilization of the triblock copolymer and TMB, with partial carbonization. Complete carbonization and silanol condensation occur slowly between 330 and 900 °C (Δm = 4.1%). Isothermal treatment at 540 °C for 60 min (Fig. 1) eliminates completely the template, resulting in the large pore FDU-1 material, ready for the enzyme immobilization.

Small angle X-ray scattering patterns for as-synthesized and calcined FDU-1 samples are shown in Fig. 2. The small angle X-ray data of the FDU-1 samples showed reflections that are indexed as (1 1 1), (2 2 0) and (3 1 1) of the face-centered cubic (fcc) structure. The small angle X-ray data patterns of these samples were quite similar to the ones reported earlier for FDU-1, synthesized by hydrothermal treatment with conventional heating [30] and microwave oven [31–34]. The interplanar spacing (d_{hkl}) and the lattice parameter (a_{hkl}) were calculated for the (1 1 1), (2 2 0) and (3 1 1) reflections. The SAXS patterns showed that the calcination process induces to a higher shrinkage of the large pores of the mesoporous structure than that obtained by usual calcination processes applied to non expanded ordered mesoporous structures. [30–34,13] and proved that the use of TMB did not destroy the organized porous network.

The insertion of TMB during the synthesis caused an average increase of 2 nm in pore size in relation to conventional synthesis carried out under similar conditions, but without the use of swelling agent (Fig. 2).

Nitrogen sorption isotherm of the analyzed large pore FDU-1 sample is shown in Fig. 3. A type IV isotherm, characteristic of materials with large mesopores and, type 2 of hysteresis loop were obtained. The latter is characteristic of samples with constricted mesopores or with pore openings smaller than the maximum diam-

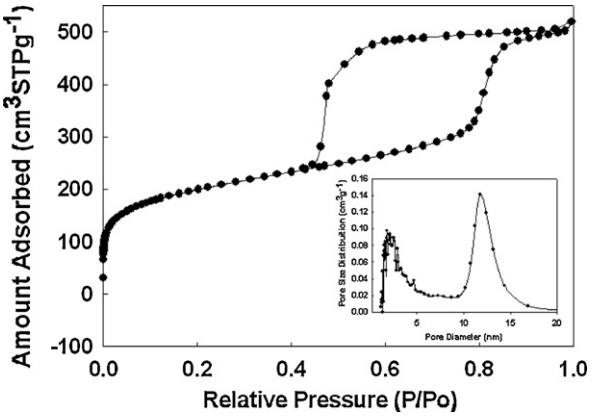


Fig. 3. Nitrogen adsorption–desorption isotherms at −196 °C for the FDU-1 sample. The inset shows the pore size distributions calculated from the isotherm.

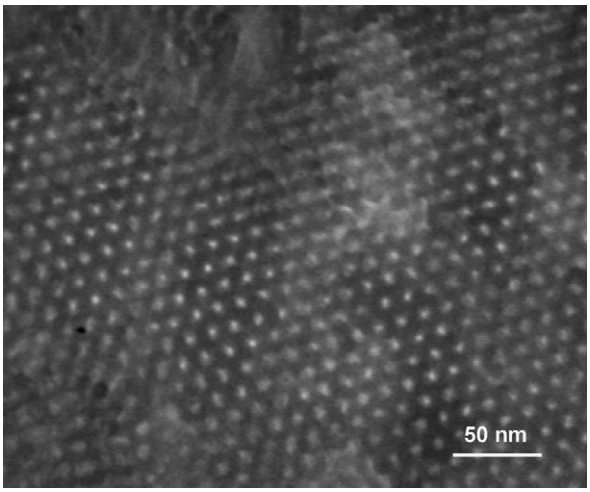


Fig. 4. Transmission electron microscopy image of the FDU-1 sample.

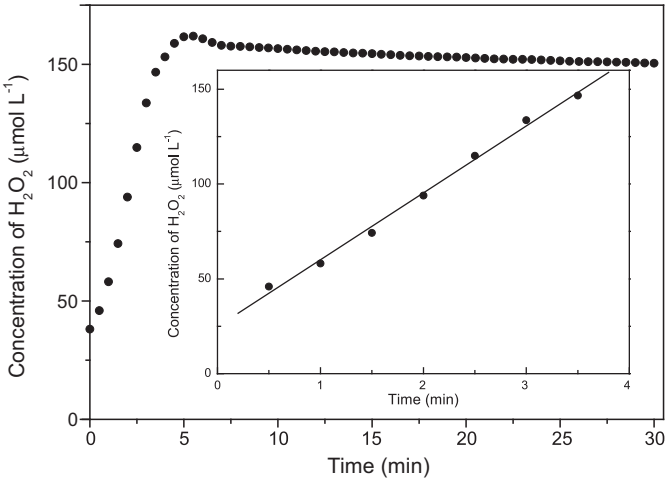


Fig. 5. Concentration of H_2O_2 as a function of time measured by molecular absorbance. spectrophotometry. The inset shows the linear tendency for first instants of reaction.

eter of the mesopores (cage-like pores) [30,31]. Also, the sample exhibited a high BET specific surface area of 684 $m^2\,g^{-1}$, total pore volume of 0.89 $cm^3\,g^{-1}$, external surface area of 49 $m^2\,g^{-1}$ and microporous volume of 0.09 $cm^3\,g^{-1}$. Its adsorption capacity and the position of the capillary condensation step were similar to those commonly observed for FDU-1 silica [30,31]. The sharp capillary condensation step at relative pressure of approximately 0.8 indicates large mesopores. The observed broad hysteresis loop, which closes at a relative pressure of approximately 0.45, is characteristic of cage-like mesoporous materials such as FDU-1 silica, having a large difference between the mesopore diameter and the entrance size of the pore [30]. The relatively small amount of micropores is located at the interconnections of the mesopores as well as to the microporosity present in the walls of cages. From the analysis of the pore size distribution (PSD), shown in the inset of Fig. 3, the mean pore size of 11.7 nm was determined.

FDU-1 silica was studied by transmission electron microscopy (TEM). The TEM micrograph in Fig. 4 reveals that the FDU-1 sample possesses a high symmetric cubic mesoporous arrangement.

3.2. Enzymatic activity determination

The absorbance of the colorimetric reagent as a function of time is presented in Fig. 5. Considering the Lambert–Beer law: $A = \varepsilon bc$, where: A = absorbance, b = optical path and ε = molar absorptive. For this case: $b = 1$ cm and $\varepsilon = 0.83\ L\,mol^{-1}\,cm^{-1}$ is possible calculated the H_2O_2 concentration. The curve of H_2O_2 concentration vs. time was plotted in the inset of Fig. 5, considering the first points of the curve, which fitted the equation: $C_{[H_2O_2]} = (25 \pm 3) + (35 \pm 1)t$, $R = 0.9974$. Thus, the enzymatic activity of GOD solution, calculated as the slope of the kinetic curve, resulted 35 ± 1 U. Spectrophotometric determination of enzymatic activity in the supernatant solution resulting from the GOD immobilization as well as the activity of the immobilized GOD on the FDU-1 silica was not possible because of the adsorption of dye solution in fragments of silica and scattering of radiation beam.

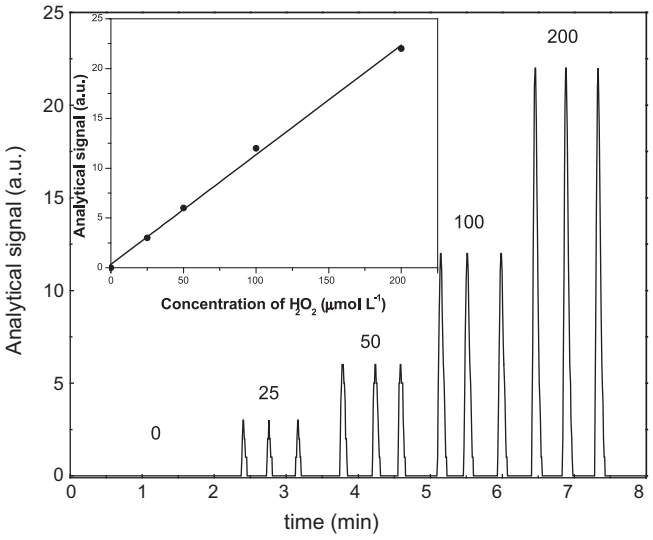
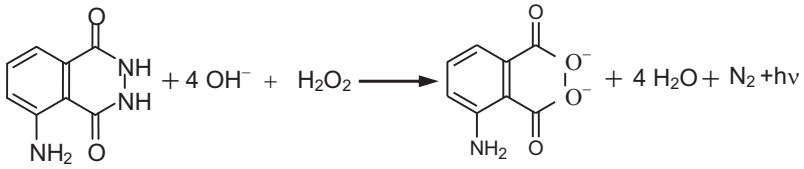


Fig. 6. Transient chemiluminiscence signals. The inset shows the analytical curve, with the analytical signal corresponding to the readings at peak maximum.

In this case the peroxide produced in the enzymatic reaction oxidizes luminol in buffered media, emitting radiation proportional to peroxide concentration, which is directly related to oxidation of glucose by O_2, catalyzed by GOD (1):



(1)

The transient signals obtained with reference standard solutions of H_2O_2 are shown in Fig. 6 together with the respective analytical curve. Linear response was observed in the range of 25–200 μmol L^{-1} of H_2O_2, obeying the equation: $I = (0.110 \pm 0.003)\,C + (0.4 \pm 0.3)$, where: I = intensity and C = concentration. Fig. 7 shows the variation of H_2O_2 concentration vs. time for the enzyme immobilized on the large pore FDU-1 silica, for the stock solution of GOD, and for the supernatant solution of GOD resulting from the immobilization process, whose first points fitted the linear equations: $I = (14.0 \pm 0.3) + (9.01 \pm 0.07)t$, $R = 0.9999$,

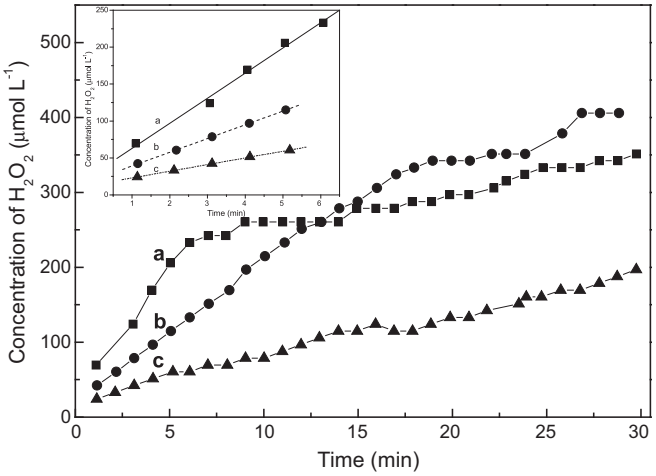


Fig. 7. Curve of H_2O_2 concentration as function of time measured by the FIA-CL method. The inset shows the linear tendency for the first instants of reaction. (a) GOD solution, (b) GOD supernatant solution, and (c) GOD immobilized on the FDU-1 silica.

$I = (29 \pm 7) + (34 \pm 2)t$, $R = 0.9968$, $I = (20.5 \pm 0.4) + (18.6 \pm 0.1)t$, $R = 0.9999$, respectively. The enzyme activity for GOD immobilized on the FDU-1 silica, GOD supernatant and GOD solution considering the equations obtained for the curves of the Fig. 7, where estimated as 9.01, 18.6 and 34 U, respectively. The value obtained for the activity of the GOD solution by FIA-CL is in agreement with that obtained by spectrophotometry with the reagent o-dianizidine.

4. Conclusion

In this article large pore FDU-1 was prepared and characterized. GOD was immobilized in this silica and catalytic tests gave positive results. The small angle scattering data of the FDU-1 samples showed reflections that were indexed as (1 1 1), (2 2 0) and (3 1 1) of the face-centered cubic (fcc) structure. The insertion of TMB during the synthesis caused an average increase of 2 nm in pore size in relation to conventional synthesis carried out under similar condition, but without the use of micelle expander. Nitrogen adsorption isotherms of the FDU-1 sample determined the total pore volume of 0.79 $cm^3\,g^{-1}$, the micropores volume of 0.25 $cm^3\,g^{-1}$ and the pore size of 11.7 nm. The hysteresis loop of H_2 type shows a typical deformation profile characteristics of materials with larger interconnection pores compared to the usual FDU-1. The studied ordered mesoporous silica is a suitable material for GOD immobilization because it enabled free diffusion of substrates and products, a feature that is a consequence of its high surface area, uniform distribution of pore size and high adsorption capacity.

Determination of enzymatic activity by the spectrophotometric method was only possible in GOD solution. This is explained by adsorption of the dye in fragments of silica and scattering of the radiation beam in the supernatant solution and in the suspension of immobilized FDU-1 silica. Determination of GOD activity in all the samples (GOD immobilized on the FDU-1 silica, GOD in supernatant and GOD solution) was possible using the FIA-CL. This approach is an interesting alternative for direct determination of enzymatic activity of enzymes that are encapsulated or immobilized in ordered mesoporous materials, with several potential applications.

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References

[1] C.T. Kresge, M.E. Leonowics, W.J. Roth, J.C. Vartulli, J.S. Beck, Nature 359 (1992) 710.
[2] C.T. Kresge, M.E. Leonowics, W.J. Roth, J.C. Vartulli, J.S. Beck, U.S. patent 5.098.684, March 24, 1992, p. 1.
[3] D. Zhao, J. Feng, Q. Huo, N. Melosh, G.H. Fredrickson, B.F. Chmelka, G.D. Stucky, Science 279 (1998) 548.
[4] D. Zhao, Q. Huo, J. Feng, B.F. Chmelka, G.D. Stucky, J. Am. Chem. Soc. 120 (1998) 6024.
[5] C. Yu, Y. Yu, D. Zhao, Chem. Commun. 7 (2000) 575.
[6] C. Yu, Y. Yu, L. Miao, D. Zhao, Micropor. Mesopor. Mater. 44/45 (2001) 65.
[7] M. Kruk, E.B. Celer, M. Jaroniec, Chem. Mater. 16 (2004) 698.
[8] J.R. Matos, L.P. Mercuri, M. Kruk, M. Jaroniec, Langmuir 18 (2002) 884.
[9] R.M. Grudzien, B.E. Grabicka, M. Kozak, S. Pikus, M. Jaroniec, New J. Chem. 30 (2006) 1071.
[10] M. Kruk, C.M. Hui, Micropor. Mesopor. Mater. 114 (2008) 64.
[11] S.B. Hartono, S.Z. Qiao, K. Jack, B.P. Ladewig, Z. Hao, G.Q. Lu, Langmuir 25 (2009) 6413.
[12] M.E. Davis, Catal. Ind. Eng. Chem. Res. 30 (1991) 1675.
[13] L.C. Cides da Silva, L.B.O. dos Santos, G. Abate, I.C. Cosentino, M.C.A. Fantini, J.C. Masini, J.R. Matos, Micropor. Mesopor. Mater. 110 (2008) 250.
[14] H. Takahashi, T. Sasaki, C. Miyazaki, T. Kajino, S. Inagaki, Chem. Mater. 12 (2000) 3301.
[15] S. Wu, H. Ju, Y. Liu, Adv. Funct. Mater. 17 (2007) 585.
[16] Y. Wei, H. Dong, J. Xu, Q. Feng, Chemphyschem 9 (2002) 802.
[17] N. Adányi, M. Tóth-Markus, E.E. Szabó, M. Váradi, M.P. Sammartino, M. Tomassetti, L. Campanella, Micropor. Mesopor. Mater. 110 (2008) 355.
[18] C.H. Lee, J. Lang, C.W. Yen, P.C. Shih, T.S. Lin, C.Y. Mou, J. Phys. Chem. B 109 (2005) 12277.
[19] U. Künzelmann, H. Böttcher, Sens. Actuators B 39 (1997) 222.
[20] K. Wang, H. Yang, L. Zhu, J. Liao, T. Lu, W. Xing, S. Xing, Q. Lu, J. Mol. Catal. B: Enzym. 58 (2009) 194.
[21] D. Jung, C. Streb, M. Hartmann, Micropor. Mesopor. Mater. 113 (2008) 523.
[22] G. Ozyilmaz, S. Tukel, O. Alptekin, J. Mol. Catal. B: Enzym. 35 (2005) 154.
[23] H.U. Bergmeyer, Reagents for enzymatic analysis, in: H.U. Bergmeyer, K. Gawehn (Eds.), Methods of Enzymatic Analysis, vol. I, Verlag Chemie, Weinheim, 1974, p. 494.
[24] F.R.P. Rocha, O. Fatibello-Filho, O. Donizeti, Quim. Nova 27 (2004) 337.
[25] G. Ranalli, E. Zanardini, P. Pasini, A. Roda, Ann. Microbiol. 53 (2003) 1.
[26] A. Economou, P. Panoutsou, D.G. Themelis, Anal. Chim. Acta 572 (2006) 140.
[27] J.F. Diaz, K.J. Balkus Jr., J. Mol. Catal. B: Enzym. 2 (1996) 115.
[28] S. Brunauer, P.H. Emmet, E. Teller, J. Am. Chem. Soc. 60 (1938) 309.
[29] M. Kruk, M. Jaroniec, A. Sayari, Langmuir 13 (1997) 6267.
[30] J.R. Matos, M. Kruk, L.P. Mercuri, M. Jaroniec, L. Zhao, T. Kamiyama, O. Terazaki, T.J. Pinnavaia, Y. Liu, J. Am. Chem. Soc. 125 (2003) 821.
[31] M. Kruk, M. Jaroniec, Chem. Mater. 13 (2001) 3169.
[32] M.C.A. Fantini, J.R. Matos, L.C. Cides da Silva, L.P. Mercuri, G.O. Chiereci, E. Celer, M. Jaroniec, Mater. Eng. Sci. B 112 (2004) 106.
[33] L.C. Cides da Silva, G. Abate, N.A. Andrea, M.C.A. Fantini, J.C. Mazini, L.P. Mercuri, O. Olkhovyk, J.R. Matos, Stud. Surf. Sci. Catal. 156 (2005) 941.
[34] L.C. Cides da Silva, T.S. Martins, M. Santos Filho, E.E.S. Teotônio, P.C. Isolani, H.F. Brito, M.H. Tabacniks, M.C.A. Fantini, J.R. Matos, Micropor. Mesopor. Mater. 94 (2006) 92.