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Synthesis and spectral characterization of a decavanadate/chitosan complex

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A decavanadate/chitosan complex was synthesized by crosslinking chitosan with decavanadate anions at a pH of 3. The materials were characterized by Fourier transformed infrared spectroscopy (FT-IR), X-ray diffraction (XRD), X-ray photoelectron spectroscopy (XPS), absorption spectroscopy (UV-Vis), and transmission electron microscopy (TEM). The spectroscopic results indicated that the decavanadate/chitosan complex was successfully obtained as result of an ionic crosslinking of the chitosan with decavanadate anions.

Key Words: Chitosan, biomaterials, vanadium compounds, crosslinking

Introduction

Most of the biological importance of vanadium is associated with its +5 oxidation state (vanadate), known to stimulate or inhibit several enzymes, probably because of similarities between the phosphate and vanadate chemistries.[1,2] For that reason, vanadium compounds have been extensively studied as potential antidiabetic, spermicidal, and anti-HIV agents.[3] Besides this, there is a growing interest in their anticancer properties.[4–6] Although the biological effects of vanadium are generally assumed to derive from monomeric vanadate or the vanadyl cation, it was recently shown that not all effects can be attributed to the simple oxovanadate forms.[7,8] The preference of decavanadate for accumulation in mitochondria, followed by the depolarization of the cell membrane and inhibition of the mitochondrial oxygen consumption, suggests an opportunity for targeting cancer.[7,8] Moreover, it was established that nanomolar concentrations of decavanadate are sufficient to provoke this action. Such an extremely low required concentration of decavanadate is considered to be a serious advantage, because the necessary doses of vanadate and vanadyl are close to the toxic level due to their

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poor absorption from the gastrointestinal tract into the blood.[9] The administration of low doses of decavanadate requires optimal gastrointestinal absorption, which could be achieved through complexing with organic ligands. It was established that the formation of complexes with organic ligands optimizes stability under the conditions of the stomach (pH approximately 2) and the small intestine (pH approximately 7.2), and optimizes absorption by the mucosa cells and desorption into the blood.[10] Chitosan (β-(1-4)-linked D-glucosamine and N-acetyl-D-glucosamine) has the potential of serving as an absorption enhancer across the intestinal epithelial because of its mucoadhesive and permeability-enhancing properties.[11] Moreover, chitosan forms stable hydrogels in acid media as a result of the protonation of its amino groups.[12]

If it is possible to synthesize a decavanadate/chitosan complex by the crosslinking of decavanadate to the chitosan, a safe passage through the pH of the stomach and the release of the decavanadate anions in the intestine as result of the changing of the pH could be achieved. To our knowledge, there is no information in the literature about the synthesis and spectral characterization of a decavanadate/chitosan complex. In previous work, a vanadyl/chitosan complex with potential antidiabetic properties was spectrally characterized by FT-IR and Raman spectroscopy.[12] Other research has focused on the study of vanadate sorption by chitosan. Sorption isotherms performed at different pH levels demonstrated that in an acidic aqueous solution (pH = 3), the main species are decavanadate anions. They are highly attracted to chitosan in its protonized form.[13] The aim of the present study was the synthesis of a decavanadate/chitosan complex through ionic crosslinking of the chitosan with decavanadate anions, and further spectral characterization.

Experimental

Chitosan from crab shells (85% deacetylated) and sodium orthovanadate (Na_3VO_4) were purchased from Sigma-Aldrich. Homogenous chitosan solution was achieved on a hot stirrer at 80 °C by adding 1 g of chitosan to 75 mL of distilled water; the pH was adjusted to 3 by using nitric acid. With respect to the maximal formation of decavanadates,[13] Na_3VO_4 was dissolved in distilled water as 1, 2, and 3 mg/mL stock solutions, adjusting the pH to 3 with HNO_3. Indicative of the presence of decavanadate with its bright yellow-orange color,[14] 1 mL of each solution was added dropwise to 3 samples of dissolved chitosan, each 25 mL. An instant bed-formation took place. The beds interconnected speedily, forming a gel membrane. According to the concentrations used of Na_3VO_4, the samples were named CS-1, CS-2, and CS-3. At the concentration of 1 mg/mL (sample CS-1), a gel membrane was not formed; only isolated ultrafine beds were observed. At the concentration of 3 mg/mL (sample CS-3), an inhomogeneous gel with many aggregates was produced. In view of the gel structure and the fact that a low concentration of vanadium compounds is recommended, the gel membrane obtained with 2 mg/mL of Na_3VO_4 (sample CS-2) was selected. The selected gel membrane was air-dried and the obtained thin film was further spectrally characterized. For the purpose of FT-IR comparison, sample CS (pure chitosan) was studied. The FT-IR spectra were collected for disk specimens with KBr using a Bruker Equinox 55 spectrometer in the range of 4000-400 cm^{-1}. Electronic absorption spectra were taken with a Cary 100 UV-VIS spectrometer within the range of 200-800 nm. Powder X-ray diffraction spectra were collected within the range of 10°-80° 2θ with a constant step of 0.04° 2θ and a counting time of 1 s/step on a Bruker D8 Advance diffractometer with Cu Kα radiation and a Sol-X detector. The spectra were evaluated with the DiffracPlus EVA package. The X-ray photoelectron spectra were obtained using unmonochromatized Al Kα (1486.6 eV) radiation in a

VG ESCALAB MK II electron spectrometer under a base pressure of 1×10^{-8} Pa and a total instrumental resolution of 1 eV. The charging effects were corrected by using the C1s peak as a reference at a binding energy of 284.6 eV. The photoelectron spectra of C1s, O1s, N1s and V2p were recorded and corrected by subtracting a Shirley-type background and were quantified using the peak area and Scofield's photoionization crosssections. The SEM micrographs were taken with a Philips 515 instrument. To reveal the influence of the washing of sample CS2 with an alcoholic aqueous solution EPR analysis was applied The EPR spectrum was registered as the first derivative of the absorption signal with an X-band ERS-220/Q spectrometer at room temperature.

Results and discussion

UV-Vis study

With the aim of instrumentally ascertaining the presence of decavanadate species, sample CS-2 was spectrally characterized in the UV-Vis range. The first scan showed a broad, weak maximum located at 390 nm (Figure 1, black line). After dilution of the 2 mg/mL solution of Na_3VO_4 at a ratio of 1:10, a new scan revealed a weak maximum at 285 nm (Figure 1, red line). From previous studies, these bands were assigned to the $O^{2-} \rightarrow V^{5+}$ charge transfer.[15] It was established that such a charge transfer led to the red shift when the coordination of the vanadium cation changed from tetrahedral to octahedral.[14] Thus, the band at 285 nm could be assigned to the V^{5+} tetrahedrally coordinated in Na_3VO_4, whereas the band at 390 nm could be attributed to the V^{5+} octahedrally coordinated in $Na_4H_2V_{10}O_{28}$. These assignments coincided with previously obtained data[16,17] and confirmed that sample CS-2 consisted of decavanadate species.

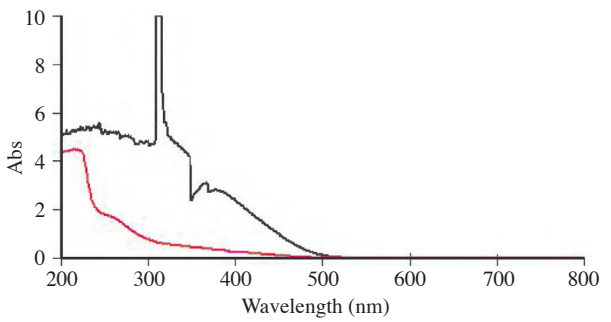

Figure 1. UV-Vis scan of 2 mg/mL solution of Na_3VO_4 (black line) and UV-Vis scan after dilution of 2 mg/mL solution of Na_3VO_4 at a ratio of 1:10 (red line).

FT-IR study

The IR spectrum of sample CS-2 (Figure 2), compared to the unprocessed chitosan, revealed a shift of the amide I band assigned to the C = O vibration of the acetamide group,[12] from 1654 to 1630 cm^{-1}. The amide II band, or the deformation vibration of the N - H bond of the amine and acetamide group,[12] disappeared at 1597 cm^{-1} and a new peak appeared at 1527 cm^{-1}. This change in wavenumbers was linked to the protonation of the chitosan amine functionalities in an acidic medium and was characterized by the presence of 2 peaks,

both attributed to NH_3^+ groups, namely the antisymmetrical deformation at 1630 cm^{-1} that overlapped the amide I band, and the symmetric deformation at 1533 cm^{-1}.[18] Thus, the established protonation of the amine group suggests the possible mechanism of chitosan gelation through electrostatic interaction with decavanadate anions. This assumption could be verified by previous studies, since a similar shift in the position of the amide I and amide II bands was reported when chitosan was chelated by vanadyl ions[12] and after phosphorylation.[18]

The presence of decavanadate anions revealed the bands indicative for the presence of polymeric vanadates. First was the vibration mode centered at 950 cm^{-1}, which was ascribed to the symmetric vibration of a V = O bond. The recorded position was about 100 cm^{-1} lower than that characteristic for the isolated vanadates.[19] This large shift may be explained in terms of oxygen bridging and V-O-V polymeric chain formation.[20] The bands located at 837, 746, and 576 cm^{-1} were characteristic of the V-O-V bridging vibration and confirmed the presence of polymeric vanadates.[14,19–21]

XRD study

XRD patterns of pure chitosan showed 2 prominent crystalline peaks at 10° 2θ and 20° 2θ.[22] In sample CS-2 (Figure 3), the crystalline peak of pure chitosan at 20° 2θ was suppressed and shifted to 22.57°, whereas another crystalline peak at 10° 2θ could hardly be seen at 12.88°. The shift and decrease in the crystallinity of each peak, as well as the appearance of a new one at 34° 2θ, suggests that the content of the decavanadate anions was modified by the chitosan matrix.

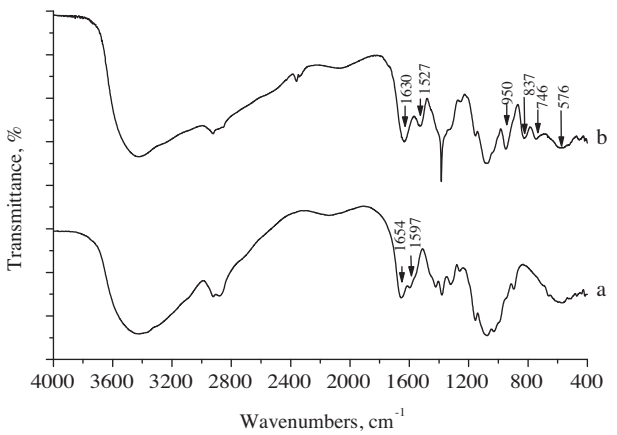

Figure 2. FT-IR spectra of samples a) CS and b) CS-2.

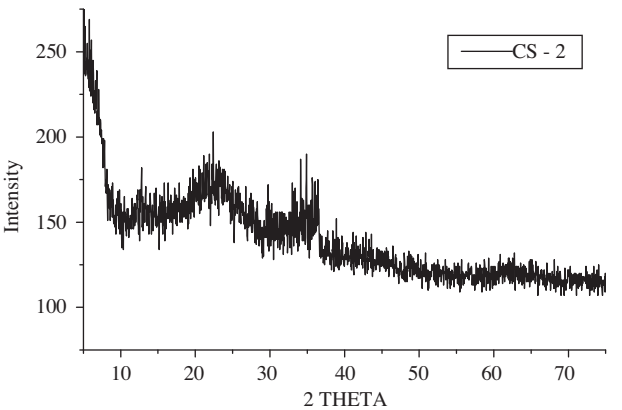

Figure 3. XRD patterns of sample CS-2.

XPS study

In sample CS-2, the deconvoluted C1s core-level spectrum consisted of 3 peaks (Figure 4). The first, situated at 284.6 eV, can be assigned to the existence of the C-C and C-N bindings. The second peak, located at 286.2 eV, was attributed to the chemical bindings of the C in C-O and C-OH bonds, and the third one, located at 288.1 eV, was related to the N-C=O chemical binding in the acetamide group.[18,23] The peak assigned to the N-C=O chemical binding was very small and indicated a small amount of N-acetylated units.

After peak fitting of the N1s core-level spectrum, 3 peaks appeared, located at 399.3, 401.6, and 406.5

eV (Figure 5). The first peak was assigned to the chemical binding of the N in the amine and acetamide groups,[18,23] while the second peak was attributed to the chemical binding of the N in the protonated amine groups.[18] There was no peak that could be linked to the protonation of the acetamide groups.[23] The third peak was assigned to the chemical binding of the N in the nitrate group.[24] The ionic binding was evidenced by the 2 amino states, i.e. a covalent nonprotonated form and an ionic protonated form. Both forms, NH_2 and NH_3^+, were likely to be present in the chitosan sample, taking into account the pKa of chitosan amine groups (approximately 6.5).[18] In a previous study, it was established that phosphorylation led to an increased number of protonated amino groups and, simultaneously, to the decrease of the N1s component at 399.8 eV.[1] Additionally, the upward shift of the electron binding energy of the peak assigned to the NH_3^+ groups, to 401.8 eV, was expected from the ionic bindings with phosphate groups in chitosan chains.[18] In sample CS-2, similar changes in the peak intensity and binding energies were observed. Thus, on the basis of the cited results, it can be assumed that ionic binding between the decavanadate anions and protonated amine groups took place.

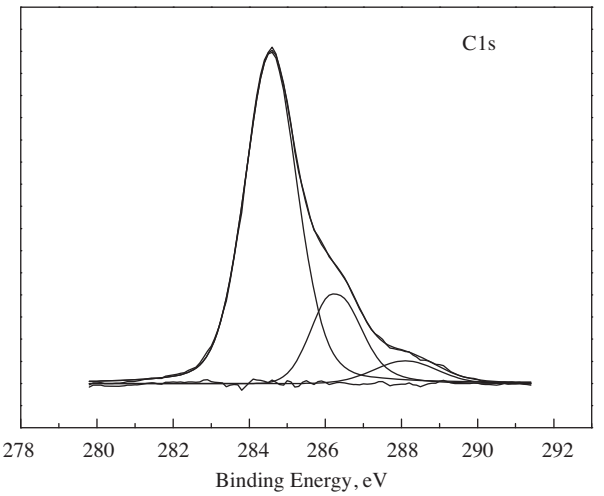

Figure 4. XPS spectrum of the C1s core-shell level of sample CS-2.

The combined signals from the V2p and O1s regions (Figure 6) are indicative of the presence of vanadium species participating in chemical bindings. The signal at 530.1 eV was attributed to the V – O chemical binding in vanadium oxides.[25] The binding energy of a $V2p_{3/2}$ peak is 516.95 eV. By using the formula $V_{ox} = 13.82 - 0.68\,(O1s - V2p_{3/2})$, the oxidation state of a chemically bonded vanadium (V_{ox}) is found to be equal to 4.85.[26] The lowering of the 5+ oxidation state was connected to the decrease of the binding energy to under 517 eV. Such a decrease is characteristic for decavanadate species and was previously observed.[27]

SEM study

The SEM micrographs in Figures 7 a and 7b present the surface morphology of the thin film from sample CS-2. An isolated crystalline phase, which is not typical for the surface morphology, can be seen. Its presence could be explained as a result of the reassembling within the sodium and decavanadate ions. The reassembling was probably the result of the excessive content of sodium orthovanadate (2 mg/mL solution, adjusted to a pH of 3).

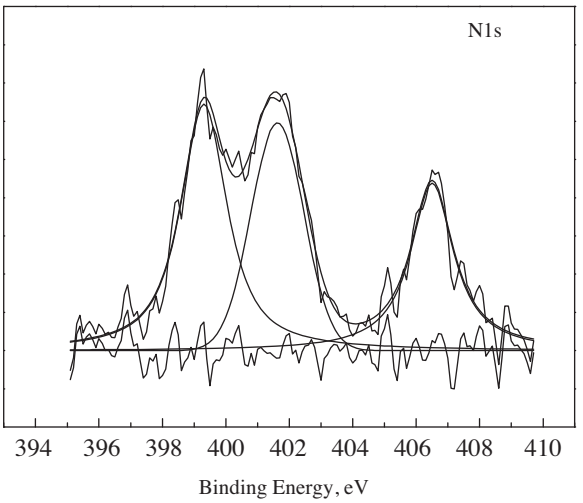

Figure 5. XPS spectrum for the N1s core-shell level of sample CS-2.

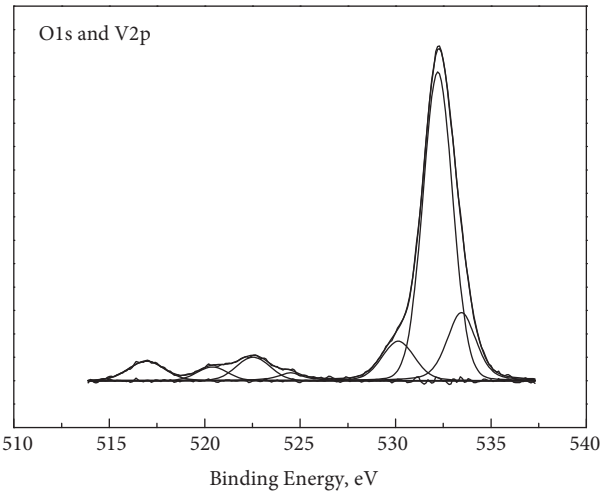

Figure 6. Combined XPS spectra of the O1s and $V2p_{3/2}$ core-shell level of sample CS-2.

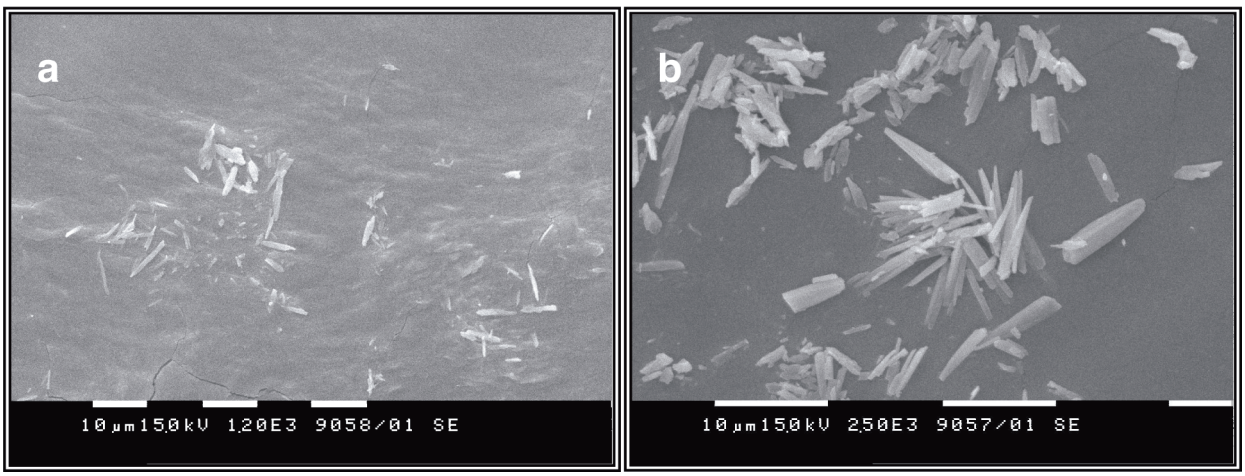

Figure 7. SEM micrographs of sample CS-2: a) $\times 1200$, b) $\times 2500$.

The observed crystals had the shape of a quadrangular prism and gave rise to the symmetry of a monoclinic system ($a \neq b \neq c$ and $\alpha = \beta = 90^\circ \neq \gamma$). The crystals that formed in the monoclinic system were characteristic, as well as the minerals (pascoite, lasalite) of the polymeric complexes, of decavanadate units.[28,29]

The lack of other crystalline forms testifies to the overall formation of the decavanadates and evidences their participation in the crosslinking process of the chitosan.

EPR analysis

With the aim of removing the crystal phase from sample CS-2, it was washed with an alcoholic aqueous solution and then examined with EPR analysis. The spectral result is presented in Figure 8. The parameters of the signal are characteristic for V^{4+} centers ($3d^1$) coupled in the vanadyl ion VO^{2+}. For comparison, the EPR

parameters of vanadyl stabilized in diammonium hydrogen citrate, $(NH_4)_2C_6H_6O_7$, are close to those obtained for the studied sample: g (perpendicular) = 2.00, g (parallel) = 1.93, A (perpendicular) = 0.0052 cm^{-1}, A (parallel) = 0.0151 cm^{-1}.[30]

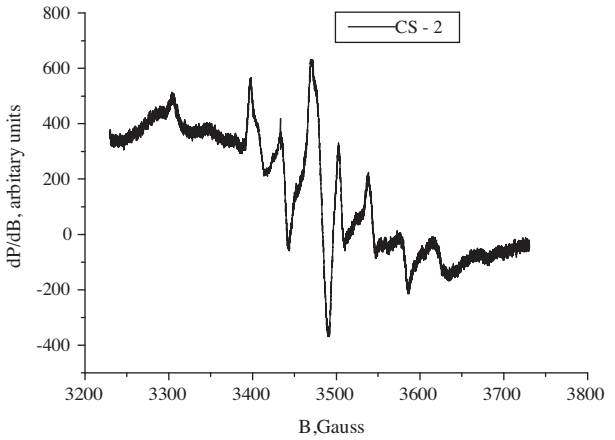

Figure 8. EPR spectrum of sample CS-2.

The characteristic 8-line signal of the V^{4+} paramagnetic centers was poorly resolved, suggesting that the paramagnetic VO^{2+} centers were not well dispersed in the sample. The detected VO^{2+} centers were probably formed as a result of the partial depolymerization of the decavanadates caused by the washing. It can be assumed that the partial depolymerization affected only the superficial layers. Further indication of this was the EPR line broadening, which is caused by the dipolar interaction of the closely situated V^{4+} centers.[31] After washing, the clustering of the V^{4+} centers[31] could occur and logically form on the surface of the sample.

Conclusions

From the collected spectral data, it could be concluded that a decavanadate/chitosan complex was successfully synthesized by an ionic crosslinking of the chitosan with decavanadate anions. Initially, the changes in the FT-IR spectrum of the studied sample, as compared to the unprocessed chitosan, contributed to this conclusion. Namely, the shift in the position of the amide I band, the disappearing of the amide II band, and the appearance of 2 novel bands assigned to the protonated amino groups support the conclusion of successful ionic crosslinking. All of these changes reveal electrostatic binding within the decavanadate anions and protonated amino groups. The XPS analysis also indicated that coupling between the chitosan and decavanadate anions occurred. The X-ray diffraction confirmed that the crystallinity of the processed chitosan exhibited a different crystalline structure than that without decavanadate anions. The SEM observations revealed that decavanadates were formed. The washing with an alcoholic aqueous solution led to the partial depolymerization of the decavanadates. Washing was probably not an appropriate approach to be used for the removing of the crystal phase. Its amount could be controlled by the fine adjustment of the sodium orthovanadate, in the range of 1-2 mg/mL.

Based on the spectral data, the possible mechanism of the crosslinking of the chitosan with decavanadate anions included an electrostatic interaction within positively charged amino groups and negatively charged

decavanadate anions. In view of the chitosan structure, the "bridge model," with the decavanadate coordinating to the protonated amine groups of 2 neighboring polymeric chains, seems to be the more possible one. By such a manner, the decavanadate anions crosslink all chitosan chains. Visually, it is displayed by the spontaneous formation of beds, followed by a fast interconnecting process within the beds and ending with gel membrane formation.

References

1. Crans, D. C. *Mol. Eng.* **1993**, *3*, 277-284.
2. Baran, E. J. *J. Braz. Chem. Soc.* **2003**, *14*, 878-888.
3. Wong, S. Y.; Sun, R. W. Y.; Chung, N. P. Y.; Lin, C. L.; Che, C. M. *Chem. Commun.* **2005**, 3544-3546.
4. Huang, C.; Zhang, Z.; Ding, M.; Li, J.; Ye, J.; Leonard, S. S.; Shen, H. M.; Butterworth, L.; Lu, Y.; Costa, M.; Rojanasakul, Y.; Castranova, V.; Vallyathan, V.; Shi, X. *J. Biol. Chem.* **2000**, *275*, 32516-32522.
5. Stanczyk, M.; Mosieniak, G.; Kaminska, B. *Neurosci. Res. Comm.* **1999**, *20*, 121-128.
6. Lei, W. H.; Liu, H. X.; Zhong, L. J.; Yang, X. D.; Wang, K. *Chinese Sci. Bull.* **2007**, *52*, 2775-27818.
7. Soares, S. S.; Gutiérrez-Merino, C.; Aureliano, M. *J. Inorg. Biochem.* **2007**, *101*, 789-796.
8. Aureliano, M.; Crans, D. C. *J. Inorg. Biochem.* **2009**, *103*, 536-546.
9. Fortoul, T. I.; Avila-Costa, M. R. *Vanadium: Its Impact on Health*, Nova Publishers, 2007.
10. Rehder, D. *Bioinorganic Vanadium Chemistry*, John Wiley and Sons, 2008.
11. Wu, Y.; Yang, W.; Wang, C.; Hu, J.; Fu, S. *Int. J. Pharmaceutics* **2005**, *295*, 235-245.
12. Baran, E. *Carbohydr. Polym.* **2008**, *74*, 704-706.
13. Guzman, J.; Saucedo, I.; Navarro, R.; Revilla, J.; Guibal, E. *Langmuir* **2002**, *18*, 1567-1573.
14. Nakamura, S.; Ozeki, T. *J. Chem. Soc. Dalton Trans.* **2001**, 472-480.
15. Ruddy, D. A.; Ohler, N. L.; Bell, A. T.; Tilley, T. D. *J. Catalysis* **2006**, *238*, 277-285.
16. Prasad, S.; Leite, V. D.; Santana, R. A. C.; Medeiros, E. A. *Ecl. Quim.* **2006**, *31*, 31-38.
17. Catana, G.; Rao, R. R.; Weckhuysen, B. M.; Van Der Voort, P.; Vansant, E.; Schoonheydt, R. A. *J. Phys. Chem. B* **1998**, *102*, 8005-8012.
18. Amaral, I. F.; Granja, P. L.; Barbosa, M. A. *J. Biomater. Sci. Polym.* **2005**, *16*, 1575-1593.
19. Fierro, L. G. *Metal Oxides: Chemistry and Applications*, CRC Press, 2006.
20. Teleb, S. M. *An. Asoc. Quim. Argent.* **2004**, *92*, 31-40.
21. Klištincová, L.; Rakovský, E.; Schwendt, P. *Inorg. Chem. Commun.* **2008**, *11*, 1140-1142.
22. Kolhe, P.; Kannan, R. M. *Biomacromolecules* **2003**, *4*, 173-180.
23. Serp, P.; Figueiredo, J. L. *Carbon Materials for Catalysis*, Wiley InterScience, 2009.
24. Liu, G.; Rodriguez, J. A.; Hrbek, J.; Dvorak J. *J. Phys. Chem. B* **2001**, *105*, 7762-7770.
25. Mendialdua, J.; Casanova, R.; Barbaux, Y. *J. Electron. Spectrosc. Relat. Phenom.* **1995**, *71*, 249-261.
26. Coulston, G. W.; Thompson, E.A.; Herron, N. *J. Catal.* **1996**, *163*, 122-129.

27. Georgiadou, I.; Papadopoulou, C.; Matralis, H. K.; Voyiatzis, G. A.; Lycourghiotis, A.; Kordulis, C. *J. Phys. Chem. B* **1998**, *102*, 8459-8468.

28. Wery, A. S. J.; Gutierrez-Zorrila, J. M.; Luque, A.; Roman, P.; Martinez-Ripoll, M. *Polyhedron* **1996**, *15*, 4555-4564.

29. Nilsson, J.; Nordlander, E.; Behrensb, U.; Rehder, D. *Acta Cryst.* **2010**, *E66*, i30-i31.

30. Karabulut, B.; Tufan, A. *Spectrochim. Acta Part A* **2008**, *69*, 642-646.

31. Typek, J.; Guskos, N.; Filipek, E.; Piz, M. *Rev. Adv. Mater. Sci.* **2010**, *23*, 196-206.