cellulose membranes had been activated by sodium periodate to form aldehide groups on their surface. Then adipic acid dihydrazide was used as a linker between cellulose dialdehyde membranes and PAA. Thus, the flux of the membranes with immobilized PAA decreased by 2.3 times in working solution with pH from 3.45 to 10. The glucose oxidase grafted to PAA by form of amid bond between amino-groups of enzyme and carboxyl groups of PAA. The transport properties of biocatalytic membranes were studied in the processes of ultrafiltration of glucose solution. The enzyme glucose oxidase catalyzes the conversion of glucose to gluconic acid and causes reduction of the solution pH. The productivity of membranes with immobilized glucose oxidase increases with time, confirming that enzyme converts glucose to gluconic acid and pH of the permeate decreases from 6.18 to 4.5.

Keywords: cellulose membrane, biocatalytic membrane, pH-sensitive membrane, polyacrylic acid, glucose oxidase.

Матеріал надійшов 11.01.2016

УДК 544.47:544.344,546.655,546.215,546.26

N. Bortnyk, A. Brichka, O. Bakalinska, S. Brichka, M. Kartel

CATALASE-LIKE ACTIVITY OF CARBON NANOTUBES SUPPORTED NANOCERIA

A series of CNT – CeO_2 nanocomposites with different nanoceria content was synthesized by reaction of cerium nitrate deposition in the aqueous media without stabilizers at room temperature. The amount of deposited cerium oxide in nanocomposite varies from 0.66 to 15.29 %. The catalase-mimetic activity of nanoceria, pristine CNT and its nanocomposites was studied in the reaction of hydrogen peroxide decomposition in the pH range of 8–11. Most of synthesized nanocomposites turned out to be effective catalysts and have a better catalytic activity than non-deposited nanoceria at all pH-values. It was established that enzyme-mimetic activity of nanoceria containing materials extremely depended on pH with the pH-optimum of 9.5–10.5. It was shown that nanocomposites with the lowest nanoceria content are more active in the reaction of hydrogen peroxide decomposition. It can be explained by the agglomeration of the nanoparticles with the increase of ceria amount in nanocomposite that causes the surface area reduction and decrease in the surface Ce^{3+}/Ce^{4+} defects content.

Keywords: catalytic decomposition, hydrogen peroxide, nanoceria, carbon nanotubes, enzyme mimetics.

Introduction

Enzymes have numerous applications in pharmaceutical [1], food, textile, and other industries [2]. However, a number of disadvantages, such as instability under environment changes (since all enzymes are proteins, they can be easily denatured when temperature or pH varies), time-consuming and expensive preparation, and inconvenience of homogeneous catalysis induced the exploration of the alternative materials [3]. As a result, a variety of materials (cyclodextrins, metal complexes, biomolecules, polymers etc.) that possess enzyme-like properties but can overcome the disadvantages of natural enzymes were discovered [4]. Different nanomaterials have also been proved to exhibit enzyme mimetic activity, among them noble metal nanoparticles [5], carbon nanomaterials (graphene oxide [6], fullerene derivatives [7], carbon nanotubes [8]), metal oxide nanoparticles such as Co_3O_4 [9], Fe_2O_3 [10], V_2O_5 [11], NiO [12], CeO_2 [13], and many other materials that are usually called artificial enzymes or enzyme mimetics.

Cerium oxide nanoparticles (nanoceria) show high catalytic activity due to the presence of surface defects (primarily oxygen vacancies) and the ability of cerium to exist in two oxidation states Ce^{3+} and Ce^{4+} (switching between them depending upon the environment) [4]. Thus, CeO_2 nanoparticles are widely used as oxidation catalyst, low-temperature gas shift catalyst, as catalyst for photocatalytic oxidation of water, for selective hydrogenation catalysis of unsaturated compounds, and many other processes [14]. Besides, nanoceria has been reported to exhibit superoxide dismutase-like [15], oxidase-like [16], and catalase-like [17] activity under different conditions.

Catalases are the class of enzymes that catalyze hydrogen peroxide decomposition to molecular oxygen and water [18]. They are present in all aerobic and many anaerobic organisms for the purpose of preventing oxidative damage that can be caused by hydrogen peroxide, which is a byproduct of a normal oxygen metabolism [9]. Besides, catalases have commercial application, for example, they are used for the elimination of residual hydrogen peroxide in textile and food industries [19].

The purpose of this work is to study catalase-like activity of nanoceria supported on carbon nanotubes (that are also known to have catalase-like properties along with stability under aggressive conditions and rather high surface area to volume ratio [20]) depending on the pH of the environment.

Materials and methods

Multi-walled carbon nanotubes (Nanothinx S. A.) with the diameter of 12–31 nm, number of layers – 15–35, and purity – 97 % (nearly 2 % – catalyst and less than 1 % – pyrolytic carbon) have been used to produce a series of nanocomposites with different nanoceria content. CNT modification can be described by the following reaction [21]:

$$4Ce(NO_3)_3 + 12NaOH + O_2 = \\ = 4CeO_2 + 12NaNO_3 + 6H_2O.$$

For preparation of nanocomposites with different content of nanoceria the calculated amount of 0.5 M $Ce(NO_3)_3$ solution (0.2; 0.7; 1.2; 3.2 and 5.8 ml) and 10 ml of distilled water was added to 2 g of CNT. Then 0.4; 1.1; 1.9; 5.0; and 9.1 ml of 1 M NaOH was added to the mixtures while stirring until the pH level was 8–9. The solid phase was filtered, rinsed and dried at 383 K. As a result, five nanocomposites with different nanoceria content were prepared. The synthesized samples were named by the estimated amount of nanoceria in it: CNT–1CeO_2, CNT–3CeO_2, CNT–5CeO_2, CNT–12CeO_2, CNT–20CeO_2.

The morphology of the nanomaterials studied by transmission (TEM, Hitachi H-800 instrument) and scanning electron microscopy (SEM, MIRA3 LMU, TESCAN instrument with a resolution of 1 nm). The content of cerium oxide in nanomaterials was determined by atomic emission spectrometry (ICPE9000 device, Shimadzu). Optical properties of nanomaterials in the UV, visible and near-IR were recorded on an UV-VIS-NIR-spectrometer UV-3600, Shimadzu in diffuse reflection mode in the 220–800 nm diapason with the uncertainty of ± 0.25 nm. Nanomaterials IR spectra were registered at room temperature on spectrometer Thermo Nicolet IR Nexus FT-IR in 5000–400 cm^{-1} diapason.

The catalytic catalase-mimetic activity of nanocomposites, pristine CNT, and nanoceria was studied in the reaction of hydrogen peroxide decomposition that can be described by following overall reaction:

$$H_2O_2 \rightarrow 1/2O_2 + H_2O.$$

To obtain the kinetics data, the volume of released oxygen was measured using microburette (volumetric method). The experiment was conducted in the H_2O_2 concentration range of 1–10 % prepared from 50 % aqueous solution of H_2O_2 at room temperature and pH range from 8 to 11 that was created by the borate buffers. Hydrogen peroxide concentration was determined using permanganatometry method. Kinetic experiment lasted for 30 min while the reaction mixture with total volume of 25 ml was continuously stirring.

The enzyme-mimetic activity of the materials was evaluated using the Michaelis constant (K_m) that was calculated from Lineweaver – Burke plot.

Results and discussion

A series of five nanocomposites with different concentration of nanoceria was obtained. Real modifier content in the samples was determined as ash content therein (Table 1).

TEM images of CNT (Fig. 1, *a*) confirm morphological and structural properties in the certificate while images of modified CNT demonstrate the presence of decorating particles (Fig. 1, *b*, Fig. 2) with the size of 6–10 nm.

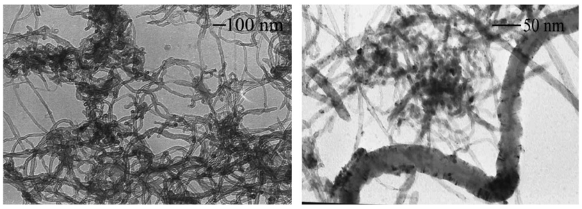

Fig. 1. TEM images of pristine (*a*) and modified (*b*) CNT

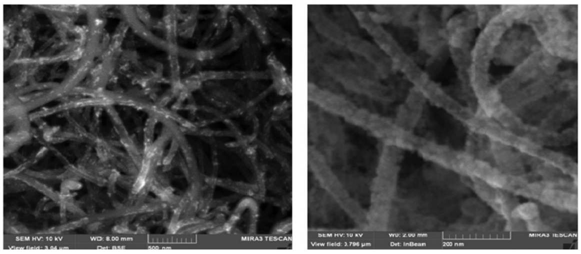

Fig. 2. SEM images of CNT–1CeO_2 (*a*) and CNT–20CeO_2 (*b*) samples

The IR spectroscopy method in the middle range of 200–4000 cm^{-1} is used to study the CNT surface modification by the atoms, molecules, functional groups [22]. CNT does not have a constant static dipole moment, their absorption bands are caused by the weak dynamic induced dipole moments. Functional groups and bonds with non carbon atoms of –COOH, –OH, C=O, –CH have strong absorption bands that can disappear just after the high temperature treatment of CNTs in a high vacuum. The obtained samples IR spectra turned out to be uninformative as fare as nanotubes strongly absorb infrared rays and are poorly polarizable structures. There is no chemical interaction between cerium oxide and carbon nanotubes, which affect the vibrational spectra of nanomaterials (Fig. 3, *a*).

Experimental diffuse reflectance UV-Vis spectra of investigated nanocomposites in the coordinates of Kubelka – Munk function are shown in Fig. 3, *b*. There is ceria signal in the UV region with a maximum at 300–330 nm. The peak intensity is increased with increase in the content of cerium oxide in the composite. For CNT–1CeO_2, CNT–3CeO_2, CNT–5CeO_2 samples ceria peak observed as a weak shoulder at the background of carbon nanotubes absorption.

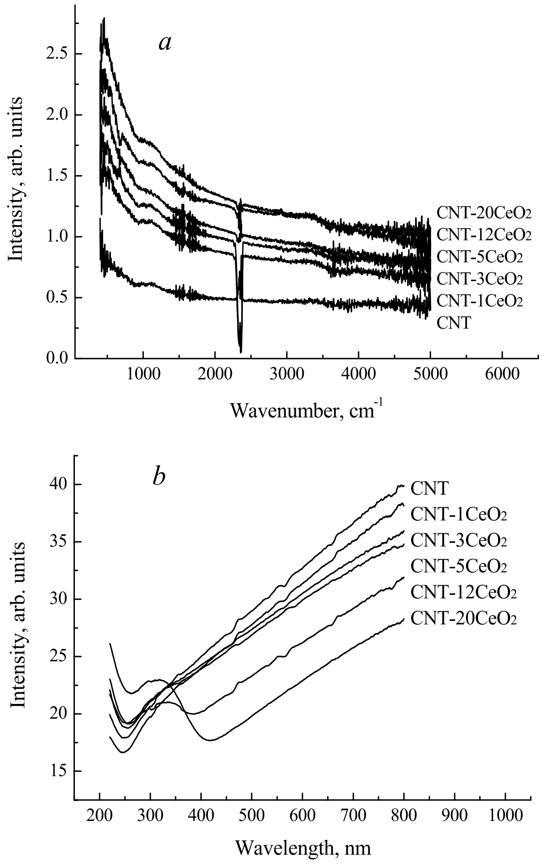

Fig. 3. Nanocomposites IR (*a*) and UV (*b*) spectra

The kinetics of the H_2O_2 (5 %, pH = 9.5) decomposition with different catalyst concentration (Fig. 4) has been investigated to determine the quantity of catalyst required for the maximal speed of the reaction.

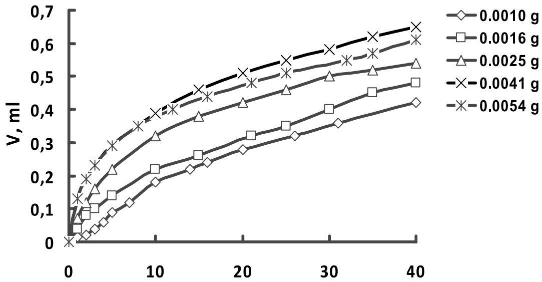

Fig. 4. Kinetics of H_2O_2 decomposition with different catalyst amounts

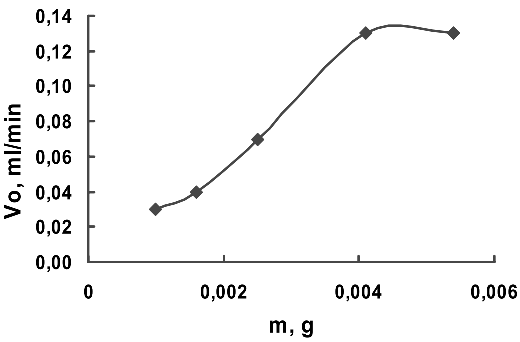

Fig. 5. Optimization of catalyst amount

This value can be found as the end of the linear part of the curve that shows the dependence of initial velocity and enzyme quantity (Fig. 5). Optimal amounts were determined for all the materials and are listed in Table 1.

the reaction rate reaches half-maximum, which means the higher Km, the lower catalyst's activity. For the ease of interpretation of experimental data, the constant of affinity ($K_{af} = 1/K_m$) was used.

Table 1. **CeO_2 content in nanocomposite and optimal amount of catalyst**

Material	CeO_2 content in nanocomposite, mass. %	m, g
CNT	–	0.0030
CNT–1CeO_2	0.66 ± 0.03	0.0050
CNT–3CeO_2	2.77 ± 0.39	0.0060
CNT–5CeO_2	3.80 ± 0.07	0.0060
CNT–12CeO_2	7.80 ± 1.78	0.0030
CNT–20CeO_2	15.29 ± 2.57	0.0040
Nanoceria	100	0.0050

Using the optimal amount of each catalyst, the kinetics of H_2O_2 (1–10 %) decomposition was investigated in order to evaluate the catalytic activity of synthesized nanocomposites. The Michaelis constants were calculated from Lineweaver – Burke plot (Fig. 6) for all the materials and are listed in Table 2.

The physical meaning of the Michaelis constant is the substrate concentration at which

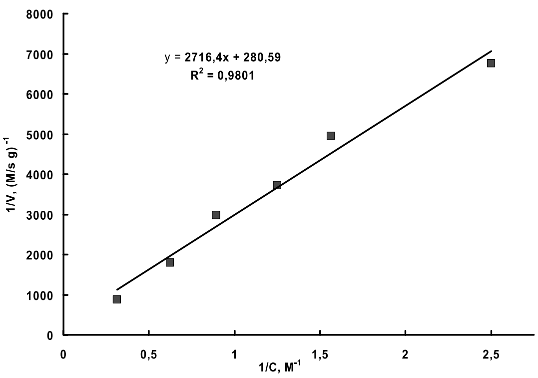

Fig. 6. Lineweaver – Burke plot for K_m calculation

The results show extremal correlation between enzyme-mimetic activity and pH-value for nanoceria and nanoceria containing nanocomposites with the pH-optimum of 9.5–10.5 (Fig. 7).

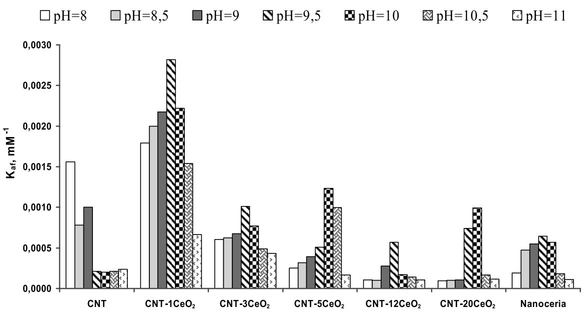

Fig. 7. Catalytic activity of the materials depending on the pH-value

Catalytic and biological activity of ceria defined by its unique redox behavior – low energy $Ce^{3+} \leftrightarrow Ce^{4+}$ transition. Ce^{3+} ions embedded in the crystal structure of cerium dioxide instead Ce^{4+} as defects. Nanoparticles of cerium dioxide, unlike oxide

Table 2. **The Michaelis constants (Km, mM) and the coefficients of determination (R^2)**

Catalyst	Km, mM; (R^2)						
	pH = 8	pH = 8.5	pH = 9	pH = 9.5	pH = 10	pH = 10.5	pH = 11
CNT	640 (0.98)	1280 (0.947)	1000 (0.96)	4710 (0.96)	4910 (0.93)	4740 (0.96)	4200 (0.97)
CNT–1CeO_2	558 (0.92)	500 (0.91)	460 (0.96)	355 (0.83)	450 (0.92)	650 (0.99)	1500 (0.91)
CNT–3CeO_2	1650 (0.99)	1600 (0.94)	1480 (0.89)	990 (0.88)	1300 (0.94)	2040 (0.98)	2320 (0.87)
CNT–5CeO_2	3980 (0.96)	2550 (0.97)	2500 (0.89)	1960 (0.96)	812 (0.87)	1000 (0.94)	6040 (0.93)
CNT–12CeO_2	9681 (0.98)	10038 (0.97)	3642 (0.97)	1763 (0.94)	5876 (0.95)	7083 (0.95)	9381 (0.97)
CNT–20CeO_2	10350 (0.99)	10025 (0.99)	9377 (0.99)	1349 (0.96)	1011 (0.98)	6094 (0.96)	8635 (0.96)
Nanoceria	5294 (0.95)	2119 (0.94)	1819 (0.92)	1558 (0.96)	1756 (0.96)	5459 (0.93)	9114 (0.99)

particles of large size, have a much larger number of surface defects. These defects are mainly surface oxygen vacancies, causing a change in the local electron and valence environment, which stabilizes the oxidation state Ce^{3+}. It was shown that nanocomposite with the lowest nanoceria content is found to be the most active in the reaction of hydrogen peroxide decomposition (Fig. 8). The decrease of catalytic activity with the growth of nanoceria content can be explained by the agglomeration of the nanoparticles that causes the surface area reduction and decrease in the surface defects – Ce^{3+}/Ce^{4+} ratio.

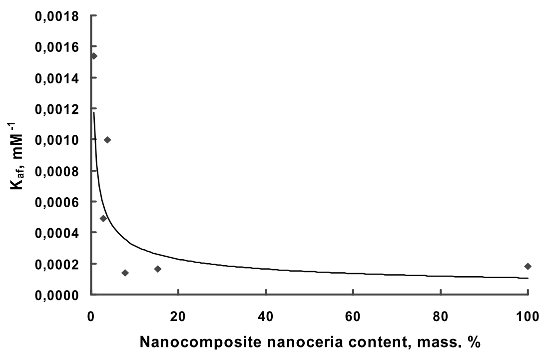

Fig. 8. Dependence of nanocomposite catalase-mimetic activity on nanoceria content under pH 10.5

Conclusions

A series of CNT – CeO_2 nanocomposites with different nanoceria content was synthesized by reaction of cerium nitrate deposition in an aqueous media without stabilizers at room temperature. Amount of deposited cerium oxide in nanocomposite very from 0.66 till 15.29 %. The catalase-mimetic activity of nanoceria, pristine CNT and its nanocomposites was studied in the reaction of hydrogen peroxide decomposition in the pH range of 8–11. Extreme dependence with the pH-optimum on 9.5–10.5 of nanoceria containing materials enzyme-mimetic activity on pH was found. It was shown that nanocomposites with the lowest nanoceria content are more active in the reaction of hydrogen peroxide decomposition. It can be explained by the agglomeration of the nanoparticles that causes the surface area reduction and decrease in the surface Ce^{3+}/Ce^{4+} defects content. Most of synthesized nanocomposites are effective catalyst, have better catalytic activity than not deposited nanoceria at all pH-values, and can be used in biotechnology for peroxide substances decomposition.

References

1. Vellard M. The enzyme as drug: application of enzymes as pharmaceuticals / M. Vellard // Current Opinion in Biotechnology. – 2003. – Vol. 14. – P. 444–450.
2. Kirk O. Industrial enzyme applications / O. Kirk, T. V. Borchert, C. C. Fuglsang // Current Opinion in Biotechnology. – 2002. – Vol. 13. – P. 345–351.
3. Research progress of nanoparticles as enzyme mimetics / X. N. Hu, J. B. Liu, S. Hou [et al.] // Science China Physics, Mechanics & Astronomy. – 2011. – Vol. 54, № 10. – P. 1749–1756.
4. Wei H. Nanomaterials with enzyme-like characteristics (nanozymes): next-generation artificial enzymes / H. Wei, E. Wang // Chem. Soc. Rev. – 2013. – Vol. 42. – P. 6060–6093.
5. Au@Pt nanostructures as oxidase and peroxidase mimetics for use in immunoassays / W. He, Y. Liu, J. Yuan [et al.] // Biomaterials. – 2011. – Vol. 32. – P. 1139–1147.
6. Graphene Oxide: Intrinsic Peroxidase Catalytic Activity and Its Application to Glucose Detection / Y. Song, K. Qu, C. Zhao [et al.] // Adv. Mater. – 2010. – Vol. 22. – P. 2206–2210.
7. A novel glucose colorimetric sensor based on intrinsic peroxidase-like activity of C_{60}-carboxyfullerenes / R. Li, M. Zhen, M. Guan [et al.] // Biosensors and Bioelectronics. – 2013. – Vol. 47. – P. 502–507.
8. Label-Free Colorimetric Detection of Single Nucleotide Polymorphism by Using Single-Walled Carbon Nanotube Intrinsic Peroxidase-Like Activity / Y. Song, X. Wang, C. Zhao [et al.] // Chem. Eur. J. – 2010. – Vol. 16. – P. 3617–3621.
9. Catalase Mimic Property of Co_3O_4 Nanomaterials with Different Morphology and Its Application as a Calcium SensorJianshuai / J. Mu, L. Zhang, M. Zhao, Y. Wang // ACS Appl. Mater. Interfaces. – 2014. – Vol. 6. – P. 7090–7098.
10. Determination of hydrogen peroxide with the aid of peroxidase-like Fe_3O_4 magnetic nanoparticles as the catalyst / Q. Chang, K. Deng, L. Zhu [et al.] // Microchim Acta. – 2009. – Vol. 165. – P. 299–305.
11. Vanadium pentoxide nanoparticles mimic vanadium haloperoxidases and thwart biofilm formation / F. Natalio, R. André, A. F. Hartog [et al.] // Nature Nanotechnology. – 2012. – Vol. 7. – P. 530–535.
12. NiO nanoparticles modified with 5,10,15,20-tetrakis (4-carboxylpheyl) – porphyrin: Promising peroxidase mimetics for H_2O_2 and glucose detection / Q. Liu, Y. Yang, H. Li [et al.] // Biosensors and Bioelectronics. – 2015. – Vol. 64. – P. 147–153.
13. Xu C. Cerium oxide nanoparticle: a remarkably versatile rare earth nanomaterial for biological applications / C. Xu, X. Qu // NPG Asia Materials. – 2014. – Vol. 6, e90. – P. 1–16.
14. Fabrication of cerium oxide nanoparticles: Characterization and optical properties / E. K. Goharshadi, K. Elaheh, S. Samiee, P. Nancarrow // Journal of Colloid and Interface Science. – 2011. – Vol. 356. – P. 473–480.
15. The role of cerium redox state in the SOD mimetic activity of nanoceria / E. G. Heckert, A. S. Karakoti, S. Seal, W. T. Self // Biomaterials. – 2008. – Vol. 29. – P. 2705–2709.
16. Evaluation of the oxidase like activity of nanoceria and its application in colorimetric assays / A. Hayat, J. Cunningham, G. Bulbul, S. Andreescu // Analytica Chimica Acta. – 2015. – Vol. 885. – P. 140–145.
17. Nanoceria exhibit redox state-dependent catalase mimetic activity / T. Pirmohamed, J. M. Dowding, S. Singh [et al.] // Chem Commun (Camb). – 2010. – Vol. 46 (16). – P. 2736–2738.
18. Nicholls P. Classical catalase: Ancient and modern / P. Nicholls // Archives of Biochemistry and Biophysics. – 2012. – Vol. 525. – P. 96–101.
19. Miłek J. Thermal stability for the effective use of commercial catalase / J. Miłek, M. Wójcik, W. Verschelde // Pol. J. Chem. Tech. – 2014. – Vol. 16, № 4. – P. 75–79.
20. Catalytic performance of carbon nanotubes in H_2O_2 decomposition: Experimental and quantum chemical study / K. Voitko, A. Tóth, E. Demianenko [et al.] // Journal of Colloid and Interface Science. – 2015. – Vol. 437. – P. 283–290.

21. Decoration of carbon nanotubes with cerium (IV) oxide / S. Ya. Brichka, I. B. Yanchuk, A. A. Konchits [et al.] // Хімія, фізика та технологія поверхні. – 2011. – Т. 2, № 1. – С. 34–40.

22. Удовицкий В. Г. Методы оценки чистоты и характеризации свойств углеродных нанотрубок / В. Г. Удовицкий // Физическая инженерия поверхности. – 2009. – Т. 7, № 4. – С. 351–373.

Бортник Н. В., Бричка А. В., Бакалінська О. М., Бричка С. Я., Картель М. Т.

КАТАЛАЗОПОДІБНА АКТИВНІСТЬ ВУГЛЕЦЕВИХ НАНОТРУБОК ІЗ НАНЕСЕНИМ НАНОРОЗМІРНИМ ОКСИДОМ ЦЕРІЮ

Ряд нанокомпозитів вуглецеві нанотрубки – CeO_2 із різним вмістом нанооксиду церію було синтезовано реакцією осадження нітрату церію у водних розчинах без стабілізатора за кімнатної температури. Кількість нанесеного оксиду церію в нанокомпозиті варіюється в межах від 0,66 до 15,29 %. Каталазоподібну активність нанорозмірного оксиду церію, вихідних вуглецевих нанотрубок та їхніх нанокомпозитів було досліджено в реакції розкладання пероксиду водню за pH 8–11. Більшість синтезованих нанокомпозитів виявляють каталітичну активність вищу, ніж ненанесений нанооксид церію за всіх pH. Знайдено екстремальну залежність від pH ензимоподібної активності матеріалів, що містять нанорозмірний оксид церію, із pH оптимумом 9,5–10,5. Показано, що нанокомпозити з меншим вмістом нанооксиду церію більш активні в реакції розкладання пероксиду водню. Це пояснюється агломерацією часток нанооксиду із зростанням вмісту оксиду, що призводить до зменшення питомої поверхні та поверхневих Ce^{3+}/Ce^{4+} дефектів.

Ключові слова: каталітичне розкладання, пероксид водню, нанооксид церію, вуглецеві нанотрубки, ензимоподібна активність.

Матеріал надійшов 25.11.2015

УДК 547.738.1:577.112.34

Михальонок Я. С., Придьма С. О., Жалніна Г. Г., Момот Л. М., Бурбан А. Ф.

НОВІ АМІНОКИСЛОТИ З СУЛЬФОНОВИМ ФРАГМЕНТОМ ЯК БУДІВЕЛЬНІ БЛОКИ ДЛЯ ТВЕРДОФАЗНОГО ПЕПТИДНОГО СИНТЕЗУ

Синтезовано нову амінокислоту з сульфоновим фрагментом – 2-аміно-2-(1,1-діоксо-1λ^6-тіолан-3-іл)-оцтову кислоту на основі тетрагідротіофен-3-ону з використанням методу Бюхерера та амінуванням основи Шиффа. Розроблену методику успішно застосовано для одержання 2-аміно-3-метансульфонілпропанової кислоти з цистеїну. Структури та чистоту нових синтезованих сульфоамінокислот встановлено за допомогою ^1H ЯМР спектроскопії та хромато-мас-спектрометрії.

Одержані амінокислоти з циклічним сульфонвмісним фрагментом є перспективними для використання як будівельні блоки у твердофазному пептидному синтезі, що відкриває шлях до розширення хімічних та фармакологічних властивостей нових сполук.

Ключові слова: амінокислоти, сульфолан, 1λ^6-тіолан-1,1-діоксид, 2-аміно-2-(1,1-діоксо-1λ^6-тіолан-3-іл)оцтова кислота, 2-аміно-3-метансульфонілпропанова кислота.