PACS: 07.07.Df, 68.43.-h

A. Tereshchenko, R. Viter, I. Konup, V. Ivanitsa, S. Geveliuk, Yu. Ishkov, V. Smyntyna

Odessa National I. I. Mechnikov University, 42, Pastera str., Odessa, 65023, Ukraine
e-mail: alla_teresc@onu.edu.ua

TiO_2-PORPHYRIN NANOSTRUCTURES FOR AMINO ACID DETECTION

A novel optical sensor based on TiO_2 nanoparticles for Valine (one of the twenty standard amino acids within proteins) detection has been developed. In the presented work, commercial TiO_2nanoparticles (Sigma Aldrich, particle size 32 nm) were used as sensor templates. The sensitive layer was formed by a porphyrin coating on a TiO2 nanostructured surface. As a result, an amorphous layer between the TiO_2 nanostructure and porphyrin was formed. Photoluminescence (PL) spectra were measured in the range of 370-900 nm before and after porphyrin application. Porphyrin adsorption led to a decrease of the main TiO_2 peak at 510 nm and the emergence of an additional peak of high intensity at 700 nm. Absorption spectra (optical density vs. wavelenght, measured from 300 to 800 nm) also showed great changes; absorption edge shift and additional peaks appearing. Adsorption of amino acid resulted in a decrease of the intensity of the PL peak due to porphyrin and an increase of intensity of the TiO_2 main PL peak. The interaction between the sensor surface and the amino acid leads to the formation of new complexes on the surface and results in a reduction of the optical activity of porphyrin. Sensitivity of the sensor with respect to different concentrations of Valine was calculated. The developed sensor can determine the consentration of Valine in the range of 0.04 to 0.16 mg/ml.

1. INTRODUCTION

Amino acids are complex molecules forming building blocks of proteins and involved in metabolism as intermediates. There are twenty amino acids involved in protein construction. Each of them contains a unique functional group, which defines the fundamental properties such as size, shape, charge, capacity for hydrogen bonding, hydrophilicity/hydrophobicity and chemical reactivity. Valine ($C_5H_{11}NO_2$), the one of the most important amino acids, is a branched-chain essential amino acid, hydrophobic and usually localized inside of proteins [1]. It is a stimulating agent which promotes muscle growth and tissue regeneration [2,3]. Valine can be used as food additive [4,5], nutrient and/or dietary supplement in animal drugs, feeds, and related products [6,7]. Because of the above mentioned properties, Valine is often used by bodybuilders (in conjunction with leucine and isoleucine) as stimulating agent. However, high concentrations of Valine can induce a crawling sensation on the skin and hallucinations [8], what is crucial for people with kidney or liver disease. Therefore, the determination of the Valine concentration in human body is an important task in medicine.

Titanium dioxide is chemically stable, nontoxic and a low-cost material which is well known for its good optical, photocatalytic and sensing properties [9-15]. Over the last decade, TiO_2 nanostructures, due to quantum-size effects such as absorption edge shift and the appearance of photoluminescence at room temperature, have been increasingly used as a sensor platform [16-26]. Recently, there has been a growing interest in the development of a new class of hybrid systems - TiO_2 -sensitizers, in which macrocycles such as porphyrins are used to form the sensitive layer [24]. Porphyrins are brightly colored pigments, containing nitrogen, that consist of conjugated multiple-loop systems, based on sixteen-membered microcycles, composed of four pyrrole molecules and bridges. A porphyrin molecule contains a coordination cavity, bound by four nitrogen atoms, having a radius of about 2Å. This molecule is capable to coordinate with metal ions of different degree of oxidation. As a result, porphyrin-metal complexes, so-called metalloporphyrins, are being formed, that possess unique combinations of structural, physical and chemical features with high biological and catalytic activity.

It is known that porphyrin application enhances photocatalytic activity of the samples. In [27], the role of both metal and macrocycle in the photocatalytic processes have been studied by utilizing TiO_2 samples coated by porphyrins and metalloporphyrins. Significant changes in optical properties of nanoporous glass filled with TiO_2 and TiO_2 /porphyrin nanostructures have previously been found [28]. In this paper we report on the investigation of new optical biosensor based on TiO_2 nanoparticles coated by porhyrin for Valine detection.

2. EXPERIMENTAL

Commercial TiO_2 nanoparticles (Sigma Aldrich, particle size 32 nm) were used as a biosensor template. TiO_2 nanoparticles were dissolved in water to prepare sols. TiO_2 layers were formed on glass substrates by dropping TiO_2 sols on the substrate and drying it at room temperature [21]. Post annealing treatment at 300 0C for 1 hour was performed to remove water from the samples. Structural properties of the obtained samples were studied by SEM.

The fabrication of sensitive layers was performed by dropping of porphyrin "5,15-di(n-nonyl),10,20-di(4-pyridyl) porphynatotin dichloride" (chemical structure is shown in Figure 1) solution in chlorophorm on TiO_2 surface. Photoluminescence (PL) spectra were measured with the setup presented in Figure 2. The samples were excited by a UV laser (LCS-DTL-374QT, L_{ex}=355 nm) and PL spectra were recorded in the wavelength range of 370-800 nm. Absorbance spectra were measured with the use of a UV-VIS spectrophotometer (Shimadzu UV-1700) in the range of 350-1100 nm.

To check the sensitivity of porphyrin to Valine, PL spectra of porphyrin layer before and after interaction with Valine were studied. To study biosensor response, different concentrations of Valine in aqueous solution were deposited on TiO_2-porphyrin surfaces.

The spectra, measured after Valine deposition, showed no drastic changes in the PL intensity and peak position (see Fig.3 in sec.).

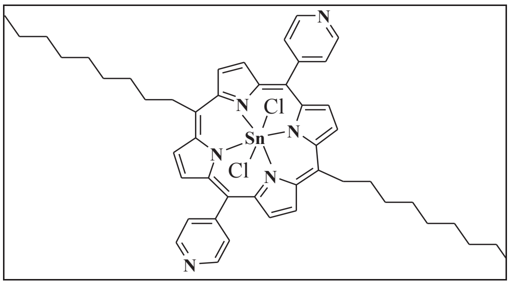


Fig.1. **Chemical structure of porphyrin**

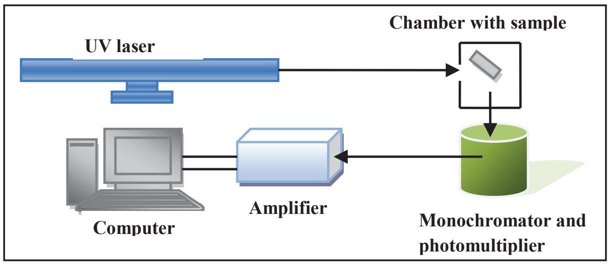
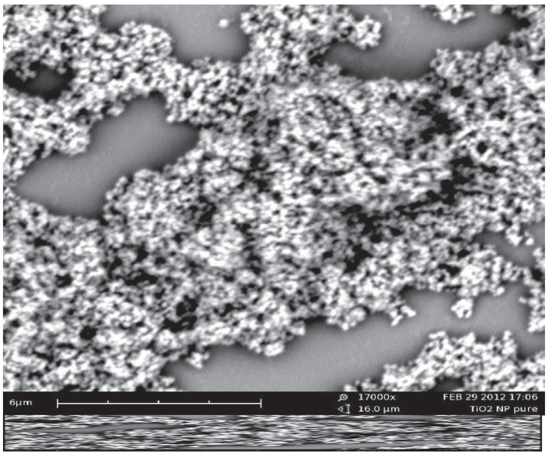


Fig.2. **Experimental setup for photoluminescence measurements**

3. RESULTS AND DISCUSSION

The obtained TiO_2 nanostructures were rough and porous as it is shown in Figure 3. Absorption spectra of initial porphyrin layer and porphyrin coated TiO_2 nanostructure are shown on Figure 4. The porphyrin demonstrated a Sorret band absorption, centered at 424 nm. It was found that after deposition of porhyrin on TiO_2, the Sorret band was shifted toward IR region, matching the interaction TiO_2-porphyrin.

Deposition of porphyrin layer resulted in significant changes in the PL spectrum of TiO_2-porhyrin nanostructure (Figure 4). Initially, TiO_2 emission spectrum showed wide peak, centered at 510 nm and the poprhyrin emission was centered at 693 nm.



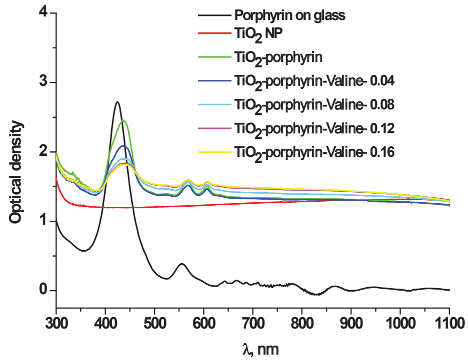


Fig.3. **SEM image of TiO_2 nanostructures**
Fig.4. **Absorption spectra of the studied samples**

The peak, related to pure TiO_2 was quenched by a factor of three, while a peak, related to porphyrin, shifted to 700 nm after the formantion of TiO_2 -porphyrin complex (Fig.5). The obtained PL data confirm the absorption results, matching to the interaction between metal oxide and porphyrin (ADD pure porphyrin spectra in the PL to discuss the changes).

We suggest that the creation of the porphyrin-metal oxide structure was caused by the formation of an amorphous layer as a result of the activation of porphyrin complexes by TiO_2. The optical properties of porphyrin could change due to a special porphyrin complex containing both hydrophobic and hydrophilic parts as well as due to labile chlorine atoms associated with the central tin atom.

Sensor response to Valine is shown in Figures 4, 5. It was found that absorption of TiO_2-porphyrin decreased with increase of Valine concentration (Figure 4).

It was found that initially, the porphyrin showed low sensitivity to Valine (Figure 5, inserted plot). The significant changes of PL intensities and peak positions observed after adsorption of Valine on TiO_2-porphyrin surface (Figure 5). Adsorption of Valine led to a quenching and a blue-shift of the porphyrin emission band. At the same time, an increase of the intensity of TiO_2 emission was observed.

The obtained results point to the irreversible interaction between porphyrin and amino acid, resulted in the formation of new complexes between them and a reduction of optical activity of porphyrin.

The sensor signal was calculated using photoluminescence and absorption data S_{lumin} (and S_{ads}):

$$S = \frac{S_0 - S_{Val}}{S_0}, \qquad (1)$$

where S_0 and S_{Val} are PL (and absorption) signals of TiO_2-porphyrin nanostructure related to porhyrin emission and absorption, measured before and after Valine adsorption, respectively. The sensitivity of the sensor was obtained as the ration of the sensor response S_{lumin} (and S_{ads}) due to (1) to the corresponding concentration of amino acid[26] C.

The sensitivity of the sensor vs Valine concentration is plotted in Figure 6. The obtained TiO_2 based sensor coated by porphyrin can detect Valine in the range of 0.04 to 0.16 mg/ml.

4. CONCLUSIONS

The TiO_2 and porphyrin form stable complex, proofed by the changes of absorption and PL of the porphyrin (IR shift) after deposition on TiO_2, matching to TiO_2 -porphyrin interaction. TiO_2 nanostructure coated by porphyrin showed good properties for Valine detection. The irreversible interaction between TiO_2 -porphyrin complex and Valine was confirmed by PL and absorption quenching after Valine adsorption and UV shift of PL peak position. The obtained results provide a basis for perspective applications of TiO_2 -porphyrin nanostructures for effective detection of Valine.

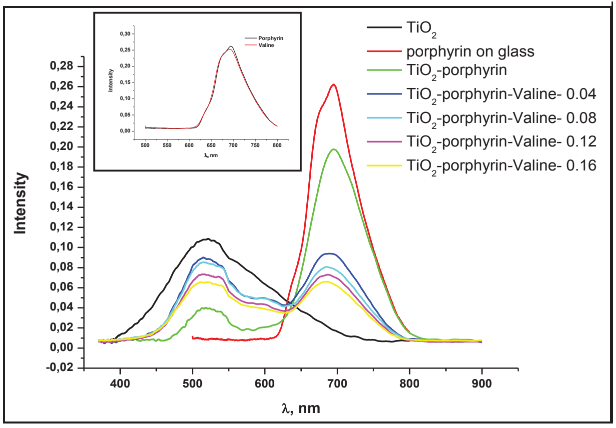


Fig.5. **PL spectra of the TiO_2-porphyrin sensor measured at**

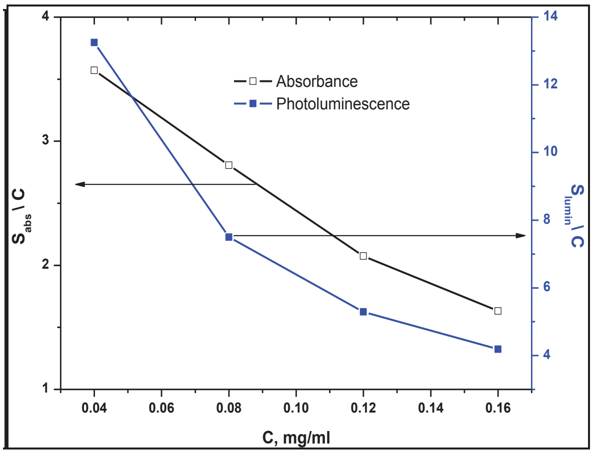


Fig.6. **Response of sensor for different concentrations different concentrations of Valine of Valine**

References

[1] Chawla, S. and Pundir, C. S., "An electrochemical biosensor for fructosylvaline for glycosylated hemoglobin detection based on core-shell magnetic bionanoparticles modified gold electrode," Biosens Bioelectron 26(8), 3438-3443 (2011).

[2] Borsheim, E., Tipton, K. D., Wolf, S. E. and Wolfee, R. R., "Essential amino acids and muscle protein recovery from resistance exercise," Am J Physiol Endocrinol Metab 283, 648–657 (2002).

[3] Volpi, E., Nazemi, R., and Fujita, S., "Muscle tissue changes with aging," Curr Opin Clin Nutr Metab Care.7(4), 405–410 (2004).

[4] Maher T. J. and Wurtmant, R. J., "Possible Neurologic Effects of Aspartame, a Widely Used Food Additive," Environmental Health Perspectives, 75, 53-57 (1987).

[5] Senyuva, H., Gokmen, V., "Interference-free determination of acrylamide in potato and cereal-based foods by a laboratory validated liquid chromatography–mass spectrometry method," Food Chemistry 97, 539–545 (2006).

[6] Fleurence, J., "Seaweed proteins: biochemical, nutritional aspects and potential uses," Trends in Food Science & Technology 10, 25-28 (1999).

[7] Tatsumi, R., "Mechano-biology of skeletal muscle hypertrophy and regeneration: Possible mechanism of stretch-induced activation of resident myogenic stem cells," Animal Science Journal 81, 11–20 (2010).

[8] http://www.anyvitamins.com/valine-info.htm

[9] Notestein, J. M., Iglesia, E. and Katz, A., "Photoluminescence and Charge-Transfer Complexes of Calixarenes Grafted on TiO_2 Nanoparticles," Chem. Mater. 19, 4998-5005 (2007).

[10] Habibi, M. H., Nasr-Esfahani, M. and Egerton, T. A., "Preparation, characterization and photocatalytic activity of TiO_2 Methylcellulose nanocomposite films derived from nanopowder TiO_2 and modified sol–gel titania," J Mater Sci 42, 6027–6035 (2007).

[11] Ding, Y., Zhang, P., Long, Z., Jiang, Y., Xu, F. and Lei, J., "Fabrication and photocatalytic property of TiO_2 nanofibers," J Sol-Gel Sci Technol 46, 176–179 (2008).

[12] Hosseinnia, A., Pazouki, M and Karimian K., "Photocatalytic reaction of aryl amines/alcohols on TiO_2 nanoparticles," Res Chem Intermed 36, 937–945 (2010).

[13] Chan, S. C and Barteau, M. A., "Physico-Chemical Effects on the Scale-Up of Ag Photodeposition on TiO_2 Nanoparticles," Top Catal 54, 378–389 (2011).

[14] Ruiterkamp, G. J., Hempenius, M. A., Wormeester, H. and Vancso, G. J., "Surface functionalization of titanium dioxide nanoparticles with alkanephosphonic acids for transparent nanocomposites," J Nanopart Res 13, 2779–2790 (2011).

[15] Qi, F., Moiseev, A., Deubener, J and Weber, A., "Thermostable photocatalytically active TiO_2

anatase nanoparticles," J Nanopart Res 13, 1325–1334 (2011).
[16] Viticoli, M., Curulli, A., Cusma, A., Kaciulis, S., Nunziante, S., Pandolfi, L., Valentini, F. and Padeletti, G., "Third-generation biosensors based on TiO_2 nanostructured films," Materials Science and Engineering C 26, 947 – 951 (2006).
[17] XiLin, X., LiXia, Y., ManLi, G., ChunFeng, P., QingYun C. and ShouZhuo, Y., " Biocompatibility and in vitro antineoplastic drug-loaded trial of titania nanotubes prepared by anodic oxidation of a pure titanium," Sci China Ser B-Chem 52(12), 2161-2165 (2009).
[18] Tasviri, M., Rafiee-Pour, H.-A., Ghourchian, H. and Gholami, M. R., "Amine functionalized TiO_2 –carbon nanotube composite: synthesis, characterization and application to glucose biosensing," Appl Nanosci 1, 189–195 (2011).
[19] Zhong, H., Yuan, R., Chai, Y., Li, W, Zhang, Y. and Wang, C., "Amperometric biosensor for hydrogen peroxide based on horseradish peroxidase onto gold nanowires and TiO_2 nanoparticles," Bioprocess Biosyst Eng 34, 923–930 (2011).
[20] Memesa, M., Lenz, S., Emmerling, S. G. J., Nett, S., Perlich, J., Müller-Buschbaum, P and Gutmann, J. S., "Morphology and photoluminescence study of titania nanoparticles," Colloid Polym Sci 289, 943–953 (2011).
[21] Viter, R., Smyntyna, V., Starodub, N., Tereshchenko, A., Kusevitch, A., Doycho, I., Geveluk, S., Slishik, N., Buk, J., Duchoslav, J., Lubchuk, J., Konup, I., Ubelis A. and Spigulis, J., "Novel Immune TiO_2 Photoluminescence Biosensors for Leucosis Detection," Procedia Engineering 47, 338 – 341 (2012).
[22] Tereschenko, A., Viter, R., Starodub, N., Ogorodniichuk Y. and Smyntyna, V., "Photoluminescence Immune Biosensor for Salmonella Detection, Based on TiO_2 Nanowires," "Photonics Technologies – Riga 2012" Programme Abstracts 48 (2012).
[23] Drbohlavova, J., Chomoucka, J., Hrdy, R., Prasek, J., Janu, L., Ryvolova, M., Adam, V., Kizek, R., Halasova, T and Hubalek, J., "Effect of Nucleic Acid and Albumin on Luminescence Properties of Deposited TiO_2 Quantum Dots," Int. J. Electrochem. Sci. 7, 1424 – 1432 (2012).
[24] Kharian, S., Teymoori, N., Khalilzadeh and M. A., "Multi-wall carbon nanotubes and TiO_2 as a sensor for electrocatalytic determination of epinephrine in the presence of p-chloranil as a mediator," J Solid State Electrochem 16, 563–568 (2012).
[25] Li, J., Kuang, D., Feng, Y., Zhang, F. and Liu, M., "Glucose biosensor based on glucose oxidase immobilized on a nanofilm composed of mesoporous hydroxyapatite, titanium dioxide, and modified with multi-walled carbon nanotubes," Microchim Acta 176, 73–80 (2012).
[26] Cosnier, S., Gondran, C., Senillou, A., Gratzel, M. and Vlachopoulos, N., "Mesoporous TiO_2 Films: New Catalytic Electrode Materials for Fabricating Amperometric Biosensors Based on Oxidases," Electroanalysis 1997, 9, No. 18 0 WILEY-VCH Verlag GmbH, D-69469 Weinheim, 1387-1392 (1997).
[27] Mele, G., Del Sole, R., Vasapollo, G., Garcia-Lopez, E., Palmisano, L., Jun, L., Slota, R. and Dyrda, G., "TiO_2-based photocatalysts impregnated with metallo-porphyrins employed for degradation of 4-nitrophenol in aqueous solutions: role of metal and macrocycle," Res. Chem. Intermed. 33(3–5), 433–448 (2007).
[28] Viter, R., Geveliuk, S., Smyntyna, V., Doycho, I., Rysiakiewicz-Pasek E. and Buk, J "Investigation of optical properties of nanoporous glass filled with TiO_2 and TiO_2/porphyrin nanostructures," PGL'2011, 32 (2011).
[29] Smyntyna V., [Semiconductor Materials for Gas Sensors], Nova Publishers, New York, 39-91 (2013)

The article is received in editorial 17.07.2013

PACS: 07.07.Df, 68.43.-h

A. Tereshchenko, R. Viter, I. Konup, V. Ivanitsa , S. Geveliuk, Yu. Ishkov, V. Smyntyna

TiO_2-PORPHYRIN NANOSTRUCTURES FOR AMINO ACID DETECTION

Abstract

A novel optical sensor based on TiO_2 nanoparticles for Valine (one of the twenty standard amino acids within proteins) detection has been developed. In the presented work, commercial TiO_2 nanoparticles (Sigma Aldrich, particle size 32 nm) were used as sensor templates. The sensitive layer was formed by a porphyrin coating on a TiO_2 nanostructured surface. As a result, an amorphous layer between the TiO_2 nanostructure and porphyrin was formed. Photoluminescence (PL) spectra were measured in the range of 370-900 nm before and after porphyrin application. Porphyrin adsorption led to a decrease of the main TiO_2 peak at 510 nm and the emergence of an additional peak of high intensity at 700 nm. Absorption spectra (optical density vs. wavelenght, measured from 300 to 800 nm) also showed great changes; absorption edge shift and additional peaks appearing. Adsorption of amino acid resulted in a decrease of the intensity of the PL peak due to porphyrin and an increase of intensity of the TiO_2 main PL peak. The interaction between the sensor surface and the amino acid leads to the formation of new complexes on the surface and results in a reduction of the optical activity of porphyrin. Sensitivity of the sensor with respect to different concentrations of Valine was calculated. The developed sensor can determine the consentration of Valine in the range of 0.04 to 0.16 mg/ml.

Key words Titanium dioxide, nanoparticles, optical sensor, porphyrin, amino acid

PACS: 07.07.Df, 68.43.-h

А. Терещенко, Р. Витер, И. Конуп, В. Иваница, С. Гевелюк, Ю. Ишков, В. Смынтына

НАНОСТРУКТУРА TiO_2-ПОРФИРИН ДЛЯ ОПРЕДЕЛЕНИЯ АМИНОКИСЛОТЫ

Резюме

Разработан новый оптический датчик, основанный на наночастицах TiO_2 для обнаружения валина (одна из двадцати стандартных аминокислот среди белков). В представленной работе наночастицы коммерческого TiO_2 (Sigma Aldrich, размер частиц 32 нм) использовались как образцы датчика. Чувствительный слой был сформирован порфириновым покрытием на наноструктурированную поверхность TiO_2. В результате, между TiO_2 наноструктурой и порфирином был сформирован аморфный слой. Спектр фотолюминесценции (ФЛ) был измерен в диапазоне 370-900 нм до и после нанесения порфирина. Адсорбция порфирина приводила к уменьшению главного пика TiO_2 при 510 нм и появлению дополнительного пика большой интенсивности при 700 нм. Спектры поглощения (зависимость оптической плотности от длины волны измеренная в интервале от 300 до 800 нм) также проявляют большие изменения; сдвиг края поглощения и появление дополнительных пиков. Адсорбция аминокислоты проявилась уменьшением интенсивности пика ФЛ обусловленная порфирином и увеличением интенсивности главного пика ФЛ TiO_2. Взаимодействие между поверхностью датчика и аминокислотой ведет к формированию новых комплексов на поверхности и является результатом уменьшением оптической активности порфирина. Была рассчитана чувствительность датчика относительно различных концентраций валина. Разработанный датчик может определять концентрацию валина в диапазоне 0.04 - 0.16 мг/мл.

Ключевые слова: Диоксид титана, наночастицы, оптический датчик, порфирин, аминокислота

PACS: 07.07.Df, 68.43.-h

А. Терещенко, Р. Вітер, І. Конуп, В. Іваниця, С. Гевелюк, Ю. Ішков, В. Сминтина

НАНОСТРУКТУРА TiO_2-ПОРФІРИН ДЛЯ ВИЗНАЧЕННЯ АМІНОКИСЛОТИ

Резюме

Розроблено новий оптичний датчик, заснований на наночастинках TiO_2 для виявлення валіну (одна з двадцяти стандартних амінокислот серед білків). У представленій роботі наночастинки комерційного TiO_2 (Sigma Aldrich, розмір частинок 32 нм) використовувалися як зразки датчика. Чутливий шар було сформовано порфіриновим покриттям на нано- структуровану поверхню TiO_2. У результаті, між наноструктурою TiO_2 і порфірином було сформовано аморфний шар. Спектр фотолюмінесценції (ФЛ) вимірювався у діапазоні 370-900 нм до і після нанесення порфірина. Адсорбція порфірина призводила до зменшення головного піка TiO_2 при 510 нм і появи додаткового піка великої інтенсивності при 700 нм. Спектри поглинання (залежність оптичної щільності від довжини хвилі вимірювана в інтервалі від 300 до 800 нм) також виявляють великі зміни; зсув краю поглинання і появу додаткових піків. Адсорбція амінокислоти проявилася зменшенням інтенсивності піка ФЛ обумовлена порфірином і збільшенням інтенсивності головного піка ФЛ TiO_2. Взаємодія між поверхнею датчика та амінокислотою веде до формування нових комплексів на поверхні і має результатом зменшенням оптичної активності порфірину. Було розраховано чутливість датчика щодо різних концентрацій валіну. Розроблений датчик може визначати концентрацію валіну в діапазоні 0.04–0.16 мг/мол.

Ключові слова: Діоксид титану, наночастинки, оптичний датчик, порфірин, амінокислота