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EVALUATION OF EFFICIENCY OF SPR-BASED BIOSENSOR AND ZnO PHOTOLUMINESCENT BIOSENSOR IN THE ANLYSYS OF BIOGENIC POLYAMINES

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ABSTRACT

Summary: In this research it was demonstrated the efficiency of the biosensors based on the different principles at the detection of the biogenic amines which are as informative elements at the diagnostic of the cancer events including development of the breast cancer. It was compared two type of the optical devices namely based on the surface plasmon resonance (SPR) and on the photoluminescence of the ZnO nanostructures. Both types of the biosensors could able to detect such low molecular agents as polyamines in model solution at the concentration from 10 to 100 ng/ml. Nevertheless SPR-based biosensor showed better results than ZnO biosensor at the detecting as low and high concentrations of polyamines. In particular, the differences in the analyse efficiency at the determination of the above indicated concentrations between the used biosensors was in range from 14% to 30%. In spite of this data it was demonstrated that ZnO biosensor also may be used for estimation of level of polyamines at the cancer diagnostics.

KEYWORDS: Biosensors, SPR, zinc oxide, photoluminescence, polyamines.

INTRODUCTION

Polyamines are promising analytical agents which can be used in many clinical test, especially it is concerned such their derivatives as: putrescine, spermidine and spermine, which are a group of naturally occurring compounds and possess a number of biological effects.[1] Chemically these compounds are organic aliphatic cations with two (putrescine),

three (spermidine) or four (spermine) amino or imino groups that are fully protonated at physiological pH values. Previous studies showed that the polyamines are closely connected with the proliferation of cells. Their biosynthesis is accomplished by a concerted action of four different enzymes: ornithine decarboxylase, adenosylmethionine decarboxylase, spermidine synthase and spermine synthase. The development and introduction of specific inhibitors to the biosynthetic enzymes of the polyamines have revealed that an undisturbed synthesis of the polyamines is a prerequisite for cell proliferation to occur. Thus, biosynthesis of the polyamines may be a meaningful indicator of certain hyperproliferative processes, most notably in case of cancer cells.[2] In this aspect the control of level of the polyamines becomes very significant at the treatment process of some cancer diseases. Especially important is express control of their changing at the appropriate process of treatment.

Methods based on the principles of biosensors are the most suitable for carrying out such express control. Among them, especially widely used in medical diagnostics are those based on the effect of SPR and the use of nanostructures of metal oxides.[3] Especially important is express control of their changing at the appropriate process of treatment.[4-5]

SPR technology for detection of a variety of disease biomarkers, hormones, and drugs at clinically relevant levels has been demonstrated very high efficiency.[6-8] We have confirmed this conclusion in case of express diagnostic of diabete, bovine viral disease und in the process of control of some toxic agents in environment.[9-12] The biosensors developed using ZnO-based nanostructures have immense potential for biomedical applications owing to their effective surface area combined with its biocompatible nature, ease of synthesis with controlled morphologies and pore sizes, and high electron communication. Further their high isoelectric point also ensures stable biomolecule immobilization while maintaining biological functionalities.[13] Functionalization with metal oxides nanostructures not only improves the biosensor device stability, but also enhances selectivity, sensitivity and lowers detection limits of the desired biosensor.[14] Nanotechnology has the potential to develop integrated electrochemical sensors with high throughput through the creation of extremely sensitive sensors such as nanorods, which has the potential of reaching a sensitivity down to a single molecule.[15] Taking into account all data about above mentioned methods on the basis of optical biosensors we curried out investigation concerning comparison of their efficiency at the detecting of polyamines.

MATERIALS AND METHODS

Procedure analysis on the SPR based biosensor.

The research was performed on a device SPR, developed at the Institute of Cybernetics of V.M. Glushkov of the National Academy of Sciences of Ukraine.

Modification of the sensitive surface of the SPR biosensor was made in the following way. As a transducer it was used a glass plate with a sprayed layer of gold with a thickness of 50 nm which was placed on a special prism in the measuring cell. At first, the measuring cell was washes by 1 ml of 0.1M phosphate-buffered saline (PBS). Next, a solution of protein A from *Staphylococcus aureus* (Sigma) at the concentration in 1 μg/ml was applied on the surface of the transducer and kept during 20 min. After thoroughly rinsing the surface by PBS and recording the change of resonance angle, the solution of monoclonal antibodies to polyamines was added to the measuring cell in a volume of 20 μl at the concentration of 100 μg/ml.Then, 20 μl of bovine serum albumin (BSA) solution at a concentration of 1 μg/ml was then applied to the surface of the transducer and after 30 min, it was washed with PBS and the resonant angle was recorded. The use of BSA is needed for the blocking of possible free spaces on the surface of the transducer, which could remain after the immobilization of specific monoclonal antibodies.

Procedure of the analysis on the biosensor based on the ZnO nanaostructures.

Modification of the transducer surface of ZnO nanoparticles was performed as following. A 5 μl solution of protein A at the concentration of 20μg/ml was applied to the surface of the ZnO nanostructures and incubated during 40 min. Then ZnO surfaces were washed with 0.85% NaCL solution. Incubation was performed in a glass or plastic Petri dish, at the bottom of which was first placed a humid filter paper on an aluminum plate. The Petri dish was placed in a refrigerator at a temperature of + 4 °C. Then, glutaric aldehyde was added in a volume of 10 μl to the plate surface and incubated for 20 min, followed by washing with 0.85% NaCL solution. After modification of the ZnO plate with the protein A, a solution of specific antibodies (obtained by immunization of rabbits with polyamines conjugated with bovine serum albumin) at the concentration of 100μg/ml and a volume of 10 μl was applied on the surface of the plates and incubated for 40 min. After that plates were washed by 0.85% NaCL solution. At last a 5 μl BSA solution in concentration of 10μg/ml was applied to the plate surface and incubated for 20 min, followed by washing with 0.85% NaCL solution.

The evaluation of polyamines was performed with the help of spectrophotometer with argon laser beam at the wavelength 380 nm. After modification surface the ZnO nanoparticles were excitated by laser and was measured the intensity of their luminesce level of which corresponds to changes of molecular weight and concentration of molecules absorbed on the surface of transducer. Intensity of luminescence fades with the addition of each new higher concentration of polyamines.

RESULTS AND DISCUSSION

At the analysis of polyamines by the biosensor based on ZnO nanostructures it was used a number of their concentrations (low and high) but the main attention was given to low ones, because at the diagnosis of cancer, such ones will have high priority. When the determining concentrations of polyamines were increased the level of the observed photoluminescence was decreased. Consequently, the attenuation of the signal indicates a gradual increase in concentration. In addition, it was observed that the determination of spermidine was clearer and better compared to spermine, however, a gradual attenuation was observed at the determination of both spermine and spermidine. It can also be seen that a clearer response of the biosensor was observed when determining the concentration of 100 ng/ml. The response level was achived 5600 photoluminescent units for spermine and 2900 for spermidine. The difference between spermine and spermidine ranged from 2300 to 3000 photoluminescent units. The difference between the samples was 500-800 photoluminescent units in spermine and 200-900 photoluminescent units in spermidine (Fig. 1).

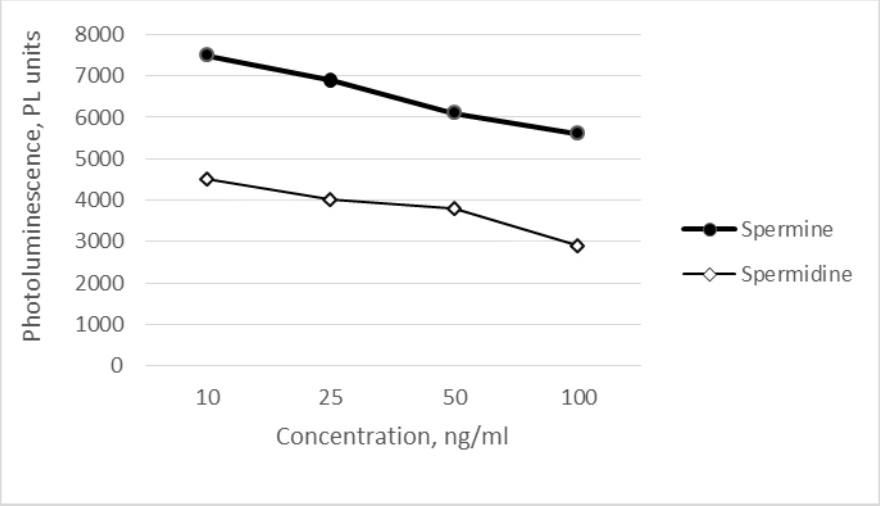

Fig. 1: Evaluation of change of photolumiscence during analysis of polyamines concentration in model solutions.

At the evaluating polyamines with help of SPR biosensor, it was determined that the resonance angle varies from 0.15 to 0.75 degrees in spermine and from 0.11 to 0.34 degrees in spermidine. The difference between spermine and spermidine ranged from 0.11 in the study of the concentration of polyamines 25 ng / ml to 0.34 degrees at a concentration of 100 ng / ml. The difference between the concentrations in the study of both spermine and spermidine is 0.2 degrees (Fig. 2).

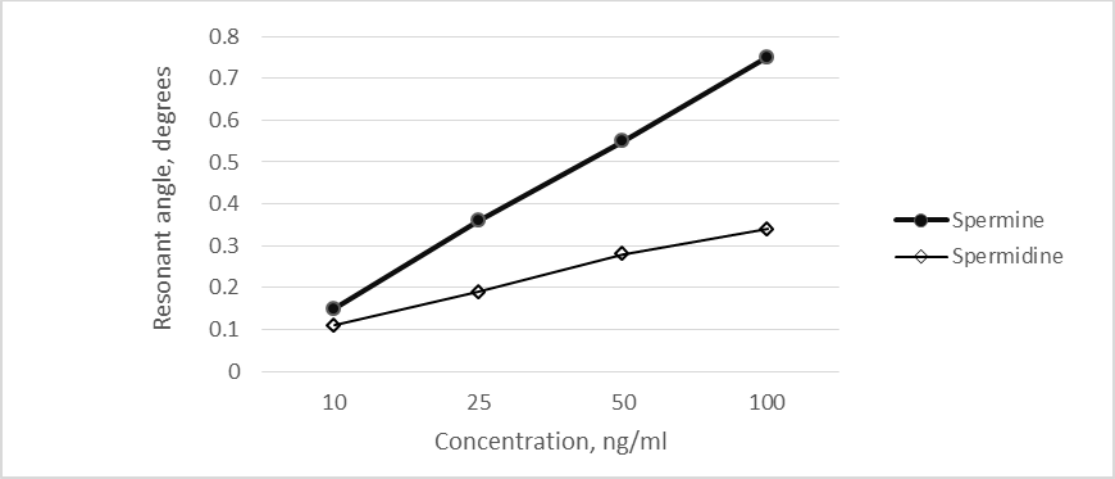

Fig. 2: Evaluation of change of resonant angle during analysis of polyamines concentration in model solutions.

Comparing the efficiency of determination of polyamines using both types of biosensors, it was determined that the photoluminescence-based biosensor performed worse than the SPR-based biosensor. Moreover, it was revealed that the SPR biosensor demonstrated more sensitivity in comparison with the biosensor based on ZnO nanoparticles by 14% in determining the concentration of polyamines at 10ng/ml. When determining the concentration of 25 ng/ml this difference was about 19%, and at 50 ng/ml it achieved 20%. This pattern is maintained with a further increasing concentration of polyamines. So, at the determination of the concentration of polyamines in the range of 100ng/ml, the biosensor based on SPR was on 30% better than the one that based on ZnO nanoparticles (Fig. 3). Moreover, the evaluation of polyamines with help of SPR biosensor is beneficial because has some useful and effective properties, like absence of necessity in some mediators or additional expensive reagents, transducer surface can be reused after cleaning and all time of analysis, in case of all preliminary preparations could take from 10 to 30 minutes of time.

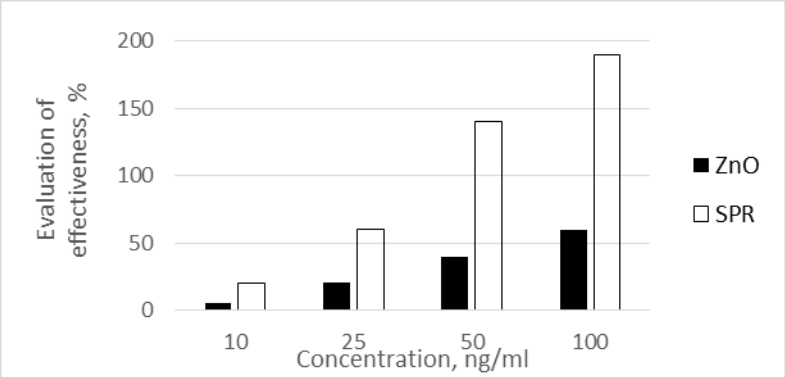

Fig. 3: Comparison of the efficiency of determination of polyamines in model solutions using biosensors based on SPR and ZnO photoluminescence.

CONCLUSION

As result of the detailed investigations we have concluded that both types of analysed optical biosensors can be recommended for practical use. Both biosensors could detect spermine and spermidine in the concentration range from 10 to 100 ng/ml.

It was found that the response of the biosensor based on the ZnO nanoparticles varies from 7500 to 2900 photo luminescent units. The difference between spermine and spermidine ranged from 2300 to 3000 photoluminescent units. Spermidine is detected better than spermine.

At the evaluating polyamines with help of SPR it was determined that the resonance angle varies from 0.11 to 0.75 degrees. The difference between spermine and spermidine ranged from 0.11 in the study of the concentration of polyamines 25 ng/ml to 0.34 degrees at a concentration of 100ng/ml. The difference between the concentrations in the study of both spermine and spermidine is 0.2 degrees.

Comparing the efficiency of the determination of polyamines it was shown that the response of the biosensor based on SPR is better then which based on ZnO nanoparticles by 14% in determining the concentration of polyamines at 10ng/ml. This different increased with the growth of the determinable level of polyamines so that when their level reaches 100 ng/ml the difference is 30%.

Comparing the results, we can say that the biosensor based on SPR was more effective than that based on ZnO nanoparticles, especially when determining high concentrations of polyamines, which makes it more effective in future use. Moreover, it is necessary to mention

that the SPR biosensor has some useful and effective properties, like absence of necessity in some mediators or additional expensive reagents, transducer surface can be reused after cleaning and all time of analysis, in case of all preliminary preparations could take from 10 to 30 minutes of time.

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