Plasmonic sensor for evaluation of the neuropeptides level in the human fluids

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Abstract

One of important problems in the diagnostic and treatment of the patient's states is operative evaluation of a type and amount of various neuropeptides secreted into the blood in human organisms. Due to similar structures and molecular weight of neuropeptides, their identification and individual evaluation is very complicated and not always consistent. For example, such substances as Oxytocin and Argenine-Vasopressin act differently on the human body, however they are look similar. The main purpose of the present work is to create a non-destructive sensor enabling operatively differentiate and evaluate the quantity of various neuropeptides existing in the human fluids such as sweat, saliva or blood. Through the experimental and theoretical efforts, it was found that the proposed goal may be solved using nanostructured semiconductor sensor producing the plasmon-polaritons in the near ultra-violet range. The frequency and intensity of generated plasmons are affected by material composition of the studied analyte. Copyright © 2018 VBRI Press.

Keywords: Oxytocin, vasopressin, plasmonic sensor, spectral characterization.

Introduction

The human organism represents a highly organized and very fine designed engineering system controlled by a plurality chemical and physical processes. Advances in biology, physics and chemistry bring up novel possibilities in diagnosis and treatment on various deviations and pathology. This way enables us more precisely effect on the various undesired possessions and increase the positive influence of medical treatment.

Oxytocin and Arginine Vasopressin are two nine amino-acides peptides with significant structural similarities, but having very different roles in the organism. Due to these similarities, vasopressin reacts sometimes with oxytocin receptors, and vice versa **[1]**. Both substances are highly multifaceted neuropeptides produced and acting within the Central Nervous System as neuromodulators, and when released into the bloodstream, as hormones, involved in regulating functioning of autonomous nervous system and several vital organs **[2,3]**. Concentrations of the peptides vary greatly between organisms, even in the same species **[4]**, in addition fluctuations within each organism **[5]**. These fluctuations within the organism have varying behavioral and psycho-neuro-immunological effects in regards to the peptides levels **[5,6]**. The structural similarities of oxytocin and vasopressin, and the similarities in pathways and influence domains create a challenge for researchers both when measuring them and their effects, and when attempting to differentiate the effects of one from the other, especially considering the gender-associated variance in the distribution of each substance **[3,7]**. The correlation and measure of OT and AVP with behavioral measures are important factors for early diagnosis and treatment of neurodevelopmental disorders with social function implications, as well as acquired conditions such as post-traumatic stress disorders, acquired anxieties, and even schizophrenia **[8,9]**. Researchers show that concentrations of mentioned neuropeptides in plasma or saliva are reliable markers of concentration in the Cerebral spinal fluid **[10-12]**, allowing extrapolating the one from the other.

Unfortunately, existing methods do not provide the possibility to reliably separate one peptide from another, as in the specific example of oxytocin and vasopressin **[13]**. Different researchers show doubt regarding the efficiency and reliability of measurement methods available today **[14]**. Thus, the development of operative reliable non-invasive method for measuring and evaluation of type and level of neuropeptides is a significant and actual task.

Last times, the plasmonic biosensors arose and compose a new group of high-sensitive optical elements. These elements are based on application of thin metal or metal-dielectric nanostructures supporting appearance of localized surface plasmons. If the basic dimensions of the metal nanostructure are smaller than the wavelength of incident light, the collective oscillation of free electrons in the metal nanostructure appears due resonant absorption of light and polarization of metal. These oscillations called as a localized surface plasmon resonance or a plasmon are defined by the shape and dimensions of the metal and by the environment of this nanostructure.

Usually, the gold and silver nanoparticles are used for implementation of plasmon-based biosensors **[15-16]**. The nanoparticles of noble metals such as gold and silver appear the plasmon absorption in the visible spectral region of light only **[17]**. However, all bodily fluids consist such component as a solution of sodium chloride in water or the saline. This substance fully absorbs all light in the infra-red range and prevents measurements of bodily fluids in the visible range. Only near ultra-violet range in the diapason 200-300 nm enables to differ and evaluate various peptides solutions with saline **[18]**. So, the plasmons generated gold and silver nanostructures are inapplicable for our goals.

It is known that plasmon may be generated only in metals with fully filled penultimate electron shell, such as this structure prevents the intraband electron transitions and enables only interband transitions. Aluminum is one of such metals. The complex nanostructures based on aluminum attract attention of researchers to these materials dues to possibility to change the wavelength of plasmon absorption and shift it in the ultra-violet direction **[19]**. One of such potentially interesting materials enabling to generate plasmons in the required optical range is an aluminum nitride (AlN) **[20]**. Thin films from AlN are known as one of the most promising piezoelectric **[21]** and electro-optic **[22]** materials.

Thin films of AlN may be grown by DC magnetron sputtering **[23]**, pulsed layer deposition **[24]**, DC/RF reactive magnetron sputtering **[25,26]**, reactive molecular beam epitaxy **[27]**, metal-organic chemical vapor deposition **[28]**. However, in our goal was to build the aluminum nanoparticles, surrounded by the AlN shell. Thus, the above-mentioned methods were considered very complex and insufficiently accurate. In this work, we present a simple method for preparation the aluminum nanostructures consisting of aluminum nanoparticles surrounded by a thin layer of aluminum nitride.

Experimental

Materials

The first task in our research was to build the nanostructured surface which may generate plasmons in the near ultraviolet range. For this goal, we chose the system consisting of small aluminum islands coated by the AlN insulating layer. The main problem in preparation of such systems is very high oxidation ability of aluminum. Therefore, all nanostructure must be grown without exposition of the aluminum islets in air. The growth process should imply two stages: growth of aluminum islets and nitridation of these islets on the low depth. From previous works with gold island thin films we know, that localized plasmon resonance appears in these nanostructures up to finishing the coalescence process **[17]**. The islands dimensions should be lower than the characteristic light wavelength. So, for creation a plasmon generating surface, we need to grow the small nanoparticles or to deposit a continuous metal thin film and produce the required structure using some lithographic process. Due to these requirements, we divided the growth process on two stages: first one is to grow the plasmon-generating nanoparticles for studying the properties of grown nanostructure, and second is preparation of a specific nanostructure with plasmon resonance properties in the range of 200-300 nm.

Thin films deposition

For creation the aluminum based nanostructures, we applied vacuum evaporation process with following plasma treatment **[29]**. **Fig. 1** represents schematically the internal view of our homemade deposition setup. This system realizes a vacuum thermal evaporation process enabling heating of the growing thin films and plasma treatment using reactive gas.

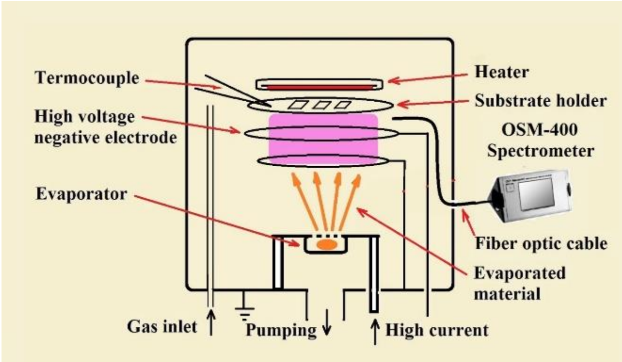


Fig. 1. Internal construction of the deposition setup.

This setup is equipped with two-stage diffusion pump based vacuum system and provides a residual pressure of (2.66–4)×10^{-6}Pa. It consists of a thermal evaporator (heated by a current of 50–120A), two ring-shaped high-voltage tungsten electrodes, a substrate holder that is situated 10–12 cm from the evaporator, and an electrical heater for the substrates. The electrical heater maintains the substrate temperature between room temperature and 250°C. The substrate temperature was monitored using a "Chromel-Alumel" thermocouple. In our experiments, we used nitrogen as a reactive gas. During the deposition process, the work pressure in the belljar was maintained through (13.3-40)Pa, providing sufficiently dense plasma with discharge currents of 1–5 mA.

Al/AlN thin film structures were grown on the glass and sapphire substrates. The substrates were cleaned in isopropanol using an ultra-sound bath. Aluminum islands were deposited by evaporation of small aluminum pieces from the molybdenum boats. A quantity of evaporated materials was calculated according the follows formulae to obtain the required thickness:

$$d = \frac{M}{2\pi\rho h^2} \quad (1)$$

where d is the thickness of the grown thin film in the center of a substrate, M is the mass of evaporated material, ρ is the density of the grown thin film, and h is the distance between evaporation source and a substrate.

Characterizations

Optical characterization of all of the films was performed in the wavelength range of 200–1100 nm using a UNICO UV-2800 UV/vis spectrophotometer and in the IR range of 2.5–5µm using a Bruker Tensor 27 FTIR spectrometer. The atomic force microscopy (AFM) images were obtained by the DI 3100 STM/AFM instrument. Sheet resistance of deposited films was measured using usual four-point probe station.

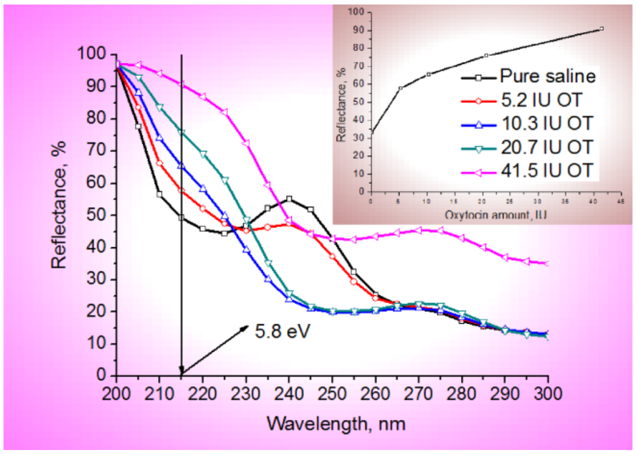


Fig.2. Reflectance characteristics of different solutions of oxytocin in saline calculated on the base of measured transmittance and absorption; The inset presents dependence of reflectance on the composition of the solution.

Results and discussion

Measurements of the reflective properties of oxytocin clearly shows that the most sensitive region for observation and evaluation of properties of various solutions containing this substance is the ultraviolet range. **Fig. 2** represents the reflectance characteristics calculated on the base of transmittance and absorption characteristics measured on the series of solutions of oxytocin in saline.

The inset in the **Fig. 2** presents dependence of reflectance on the composition of the solution. As shown, the reflectance is an unambiguous function of the oxytocin concentration in saline. The various solutions of vasopressin in saline show the same behavior, however a function looks a little bit another. Such performance of different neuropeptides in saline and possibility to differ them in the ultraviolet range of light were the reason to build the sensor that may be sensitive in this light-diapason and can produce plasmon generation for sensitivity increasing.

Fig. 3 represents an aluminum islands thin film grown on the glass and their transmittance. This film, immediately after deposition of aluminum, was heated for temperature of 200^{0}C during 15 min under nitrogen plasma treatment with the plasma current of 3.2 mA.

As shown in **Fig. 3,** the islands thin-film system Al/AlN grown on glass represents a nanostructure with average heights of islands approximately 5 nm and radius of about 50-70 nm. Such system appears the plasmon resonance absorption in the wavelength of ~500 nm.

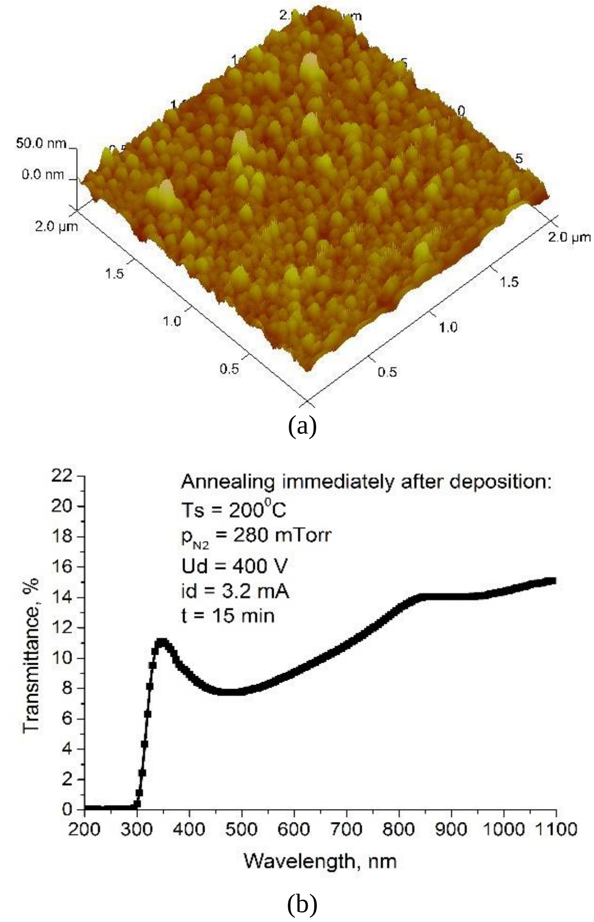


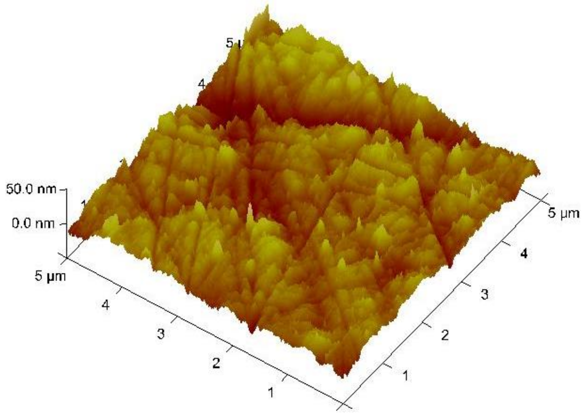
Fig. 3. AFM presentation of the islands Al/AlN thin-film system grown on the glass substrate (a) and their transmittance (b).

In accordance with the capabilities of our measurement equipment, we have conducted measurements of transmittance and absorption of thin-film systems. Thus, the samples using glass substrates cannot show the system behavior in ultraviolet range. Therefore, to provide measurements in ultraviolet, we used the sapphire substrates.

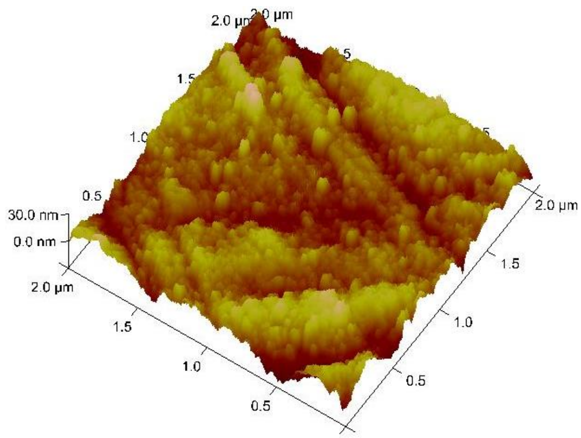
Fig. 4 represents two various Al-based thin-film systems observed using AFM microscope and their transmittance characteristics. Evidently, the sapphire surfaces are very developed due to its high hardness. However, they are enables measurements in the ultraviolet due to the high bandgap of the sapphire.

Fig. 4-a shows the external view of the complex $Al_2O_3/Al/Al_2O_3$ system where first layer of ~2 nm thick is the native oxide grown on the surface of Al islands immediately after exposition of the sample in air. The native aluminum oxide film is very dense and stable and it protect the Al islands from additional oxidation. Second figure, **4-b**, represents the $AlN/Al/Al_2O_3$ system. Here, the aluminum nitride film grown on the surface of the Al islands without exposition the grown aluminum film in air. Comparing of these surfaces, shown in **Fig. 4-a** and **4-b**, shows that the aluminum nitride coating keeps the shape of the islands Al film, while the appearance of aluminum islands after oxidation looks smoother. Another material of coating and more sharp shape of the islands bring to us

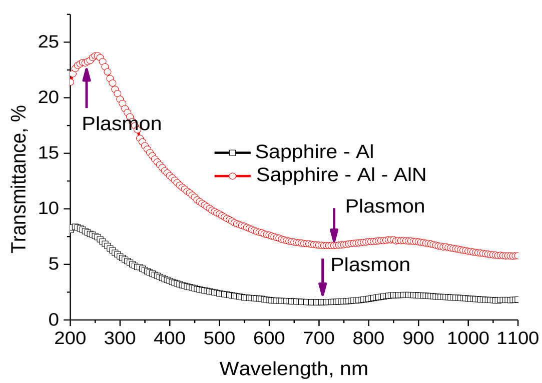
additional plasmon resonance in the ultraviolet range as shown in the **Fig. 4-c**. So, the Al/AlN structure represents two plasmon peaks, one in the visible-near infrared part of spectrum and second one in the ultraviolet wavelength.



(a)



(b)



(c)

Fig. 4. Various thin-film Al-based structures and their transmittance: (a) The islands aluminum thin film grown on the sapphire and exposed on air (Al/Al_2O_3); (b) The islands aluminum thin film grown on the sapphire and immediately treated in the nitrogen plasma under heating (Al/AlN); (c) Transmittance characteristics of both thin-films systems.

So, we obtained a coating enables to produce plasmonic absorption in the ultraviolet part of spectrum. The follows step is preparation of the sensor with the developed nanostructured coating working in the required part of spectrum. **Fig. 5** represents a schematic view on the proposed sensor and its principle of operation.

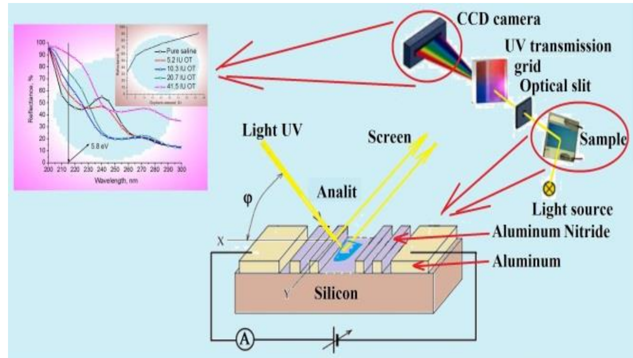


Fig. 5. Principle scheme of the proposed sensor and its principle of operation.

The sensor comprises a semiconductor (silicon) substrate and the thin-film system prepared from continuous aluminum metal film divided on the parallel strips with dimensions of 200×215 nm and with the same distance between strips. All strips also coated by the AlN film with thickness of 2-3 nm. These dimensions are due to the maximum sensitivity wavelength that was found on the studied neuropeptides. The sensor will work with the optical system which also schematically shown in **Fig. 5**.

This system works by follows: a sensor with the active surface wetted by the human fluid and dried by the air flow inserts in the system and illuminated by ultra-violet light. The light reflected from the sensor surface comprises the information about the studied substance. This light will be converted to the spectral form and directed to the CCD for the spectral picture receiving and treatment using a built-in microprocessor. The main goal of the built-in microprocessor is calculation of the optical characteristics of the studied substance, their composition and amount.

This sensor will be used for creation a mobile low-cost device for operative definition of the oxytocin or other neuropeptide in various human fluids, for example in the sweat or saliva.

Conclusion

In conclusion:

1. Novel deposition system on the base of vacuum evaporation, enabling thin films treatment in the environment of reactive-gas plasma was designed and built.
2. The Al-AlN thin film system were prepared using novel deposition setup.
3. It was found that the Al-AlN thin film system appears the surface plasmon resonance in the mid-ultraviolet spectrum range.
4. The Al-AlN thin film system deposited by plasma-enhanced evaporation method may be applied for biosensors preparation.

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