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Optical characterization of oxytocin and arginine-vasopressin dissolved in saline

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ABSTRACT

Oxytocin and arginine-vasopressin are two very important neuropeptides secreted into the blood in human organisms. They have similar structures and molecular weight, complicating their differentiation, identification and individual evaluation. The purpose of the present work is to investigate the optical properties of oxytocin and vasopressin dissolved in saline. This information can provide baseline templates for the detection of these neuropeptides in bodily liquids containing the salt, such as saliva or sweat, without the need for complex chemical isolation processes. In our work, we investigated optical properties of solutions containing different amounts of the peptides in saline. Transmittance and absorbance of these solutions were measured in a wide spectral range. Measurements were conducted at pre-determined time-intervals, allowing evaluation of decomposition time of the samples. It was found that the most representative spectral region for correct characterization of neuropeptides is the ultra-violet spectral area. Calculated optical bandgap of various peptide solutions was found to be a quality and quantity evaluation parameter, enabling differentiation of considered materials and evaluation of decomposition time. It was found that the optical bandgap value rises toward the saline with the degradation of the studied substance over time.

Keywords: *oxytocin, vasopressin, spectral characterization, optical bandgap*.

1. INTRODUCTION

The organism is a fine tuned wonder of engineering, maintained and coordinated by a great many chemical and physical processes. With the advances in biologic science, we are able to visualize and understand more and more of these. The medicinal field, especially, gains much from this progress as deeper knowledge of an organism's function allows us precise intervention in the cases of pathology or prophylactics. This gives us the ability to choose more focused treatment, reducing undesired effects, and increasing efficacy while keeping dosage to a minimum. The primary foci of this article are two chemicals with wide expression on both physiology and psychology, in humans and other organisms. Oxytocin (OT) and Arginine Vasopressin (AVP) are two nine-amino peptides with certain structural similarities, but having very different roles in the organism, across many systems. Due to the similarities between the substances, AVP is sometimes seen reacting with OT receptors, and vice versa [1]. Both substances are highly multifaceted neuropeptides produced and acting within the Central Nervous System (CNS) 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 to circadian and other fluctuations within each organism [5]. These fluctuations within the organism have varying behavioral and psycho-neuro-immunologic effects in regards to the peptides levels [5,6].

OT, commonly known as the Birth Hormone due to its role in muscle contractions and milk excretion [7], also plays a critical part in heart muscle functioning [8]. In nociceptive systems, it modulates pain sensitivity [9], and is positively involved in healing processes [10]. OT also holds fame as the "social hormone", due to the involvement in processes of psychological and physiological attachment, empathy and caring to both extents, of positive and negative [11]. In psychiatry, oxytocinergic abnormalities have been found to be involved in several social disorders. Feifel et. al (2010) claim that antipsychotic properties of oxytocin have caused reductions in schizophrenia symptoms [12]. OT may be used as a possible treatment intervention in cases of depression, anxiety [13], and Autism Spectrum Disorders (ASD) [14]. For example, an enhancement effect of OT treatment has been reported on ASD children brain function [15].

The hormone AVP regulates water retention in mammals, as well as vascular resistance, thus regulating levels of blood pressure, temperature dispersion and waste disposal [16]. When acting in the CNS as a neuropeptide, AVP plays important roles in aggressive and other social behaviors [17], reproductive behaviors and functions, as well as pair bonding and maternal behaviors [6]. The effects of AVP are notably related to physiological and social stress- and aggression-based behaviors [18], leading researchers to seek this involvement when creating treatments for abnormalities in these behaviors [19,20].

The structural similarities of OT and AVP, 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 [3,21]. 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 (PTSD), acquired anxieties, and even schizophrenia [22,23]. Researchers show that concentrations of OT and AVP in plasma or saliva are reliable markers of concentration in the Cerebral spinal fluid (CSF) [24-26], allowing extrapolating the one from the other.

Unfortunately, some researchers show doubt regarding the efficacy and reliability of measurement methods available and in use today [27]. The assessment methods, involving blood-sampling procedures need to consider the effect pain and stress have on both peptides' levels. Furthermore, both peptides have rather short lifespans, requiring quickly performing complicated assay, the reliability of which are themselves sometimes in question [28]. Young and Anderson (2010) express the opinion that peer review is not as perfect as science wishes it to be, and that many practitioners are not equipped with adequate statistical and analytical training, tools and knowledge [29]. For example, they show that interpretation of research based on correlations between chemical and behavioral measures must take into account the possibility of measurement flaws, statistic and analytical errors. Yet, issues can be a result of misuse or misinformation of measurement techniques and technology, such as using general, low sensitivity assay kits when attempting to measure low concentration peptides such as OT and AVP [29].

Development of operative and accurate methods for neuropeptide estimation requires knowledge of the substances' properties, and in particular the optical properties. In the presented work, we measure the optical transmittance and absorption of several fluid solutions of oxytocin and vasopressin in saline in a large spectral range. We believe that this information will contribute greatly to the development of a non-invasive method of neuropeptide levels assessment using saliva and other bodily fluids.

2. EXPERIMENTAL SECTION

Optical characterization of all samples was done at wavelengths of 200–1100 nm using the UV-2800 UV/VIS spectrophotometer of UNICO, and in the infrared (IR) range of 2.5-20 μm using a FTIR Tensor 27 of Bruker. Figure 1 represents the principle optical scheme used to our measurements. Liquid solutions were inserted in quartz cuvettes of UNICO to enable observation of optical characteristics in the near ultraviolet (UV) range.

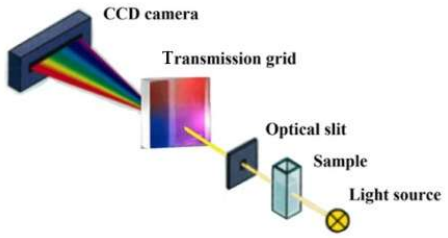

Figure 1. A principal arrangement scheme of optical measurements.

The OT and AVP were purchased from a commercial source, Sigma-Aldrich. Liquid samples were prepared by dissolving the peptides in saline (0.9 Sterile Sodium Chloride NaCl Normal Saline) in various, gradually declining concentrations. Saline was used instead of water due to the fact that NaCl is present in most bodily fluids, therefore enabling the resulting optical characteristics to be a closer match to those observable in a medical scenario, such as when measuring for OT or AVP in sweat, or CSF. Thus, saline is used in this study to represent bodily fluids, rather than water. Furthermore, being able to measure the peptides in question without physically isolating them from their environment is an important issue, thus the ability to filter out "noise" caused by the saline would serve as a proof of concept that this method is viable in medical research and applications. It is important to note, however, that more research, using different solutions, is needed to further validate the methods proposed here.

3. RESULTS SECTION

First of all, we tried to measure the transmittance of the pure saline in a large infra-red spectrum range. Figure 2 represents the transmittance characteristics of saline, soda lime glass and sapphire slides in the wide IR spectrum range. As shown in this figure, the saline completely absorbs light in the near and far infrared ranges. Therefore, all fluids containing sodium chloride, such as blood, saliva etc. will not be transparent in these wavelengths. Thus, infra-red optical spectra are not applicable for the studied neuropeptide solutions.

In light of these results, spectral analyses of most bodily fluids in the IR ranges seems to be rendered moot due to how the saline effectively blocks these wavelengths from providing any information besides the presence of said saline.

Figure 3 represents the transmittance characteristics recorded in the near ultraviolet (UV), visual and near infrared optical ranges, from 200 nm up to 1100 nm. Transmittance characteristics of pure saline and two solutions of neuropeptides in saline with equivalent substance amounts are presented here.

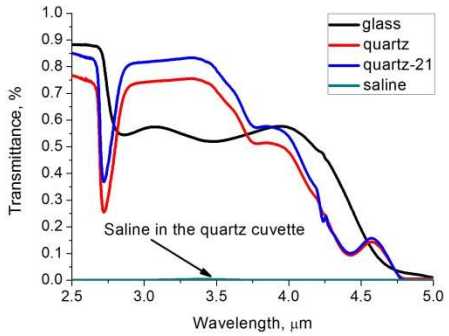

Figure 2. Transmittance of the saline and various material's slides in the IR range.

These characteristics look very similar in the visual and near infrared ranges. However, substantial differences in the

characteristic shapes appear in the mid-ultraviolet range as shown in the insertion. The thickness of all liquids is the same, 10 mm, therefore these differences are due to the different absorption properties of the two examined neuropeptides. The exposed effect of absorption in the UV range may be explained by excitation of peptides with energetic photons and increase of their internal energy in the interval of 3.76-5.90 eV which leads to electron detachment from the peptides [30]. The light wavelength suitable for this excitation is 210-330 nm, i.e. the ultraviolet range.

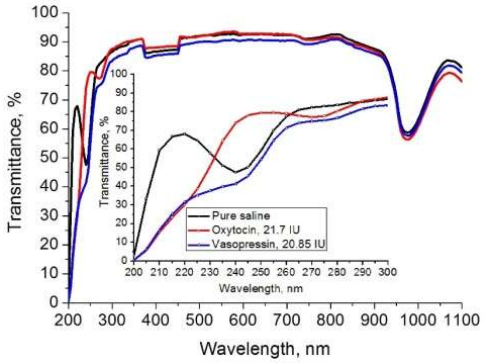

Figure 3. Transmittance of the pure saline and neuropeptides solutions in the near UV and visual optical range.

Figure 4 represents transmittance characteristics of the oxytocin solutions recorded in the mid-UV range for different amounts of the neuropeptide in the saline. Here, all curves represent interferential oscillations. Interestingly, at the 215 nm wavelength, which relates with a photon's energy of 5.8 eV, all transmittance curves became dependent on the neuropeptide amount.

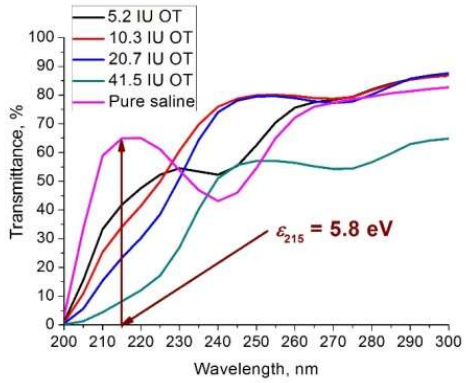

Figure 4. Dependence of the oxytocin solution transmittance on the concentration.

Similar dependence may be observed for the vasopressin solutions as presented in figure 5. At the same wavelength, the transmittance of AVP solutions depend on the amount of the studied material. Therefore, we can estimate the amount of the material using the transmittance measurement. Moreover, comparison of these dependencies enables us to differentiate one peptide's solution from another.

Figure 6 presents dependence of solution transmittance on neuropeptide amounts, as expressed in specific exponential fitting shown on both experimental results. The two graphs, OT dependence and AVP dependence, have quite similar curves, however the fitting curves have different derivatives. These derivatives may be taken as a main parameter characterizing the type of neuropeptide present.

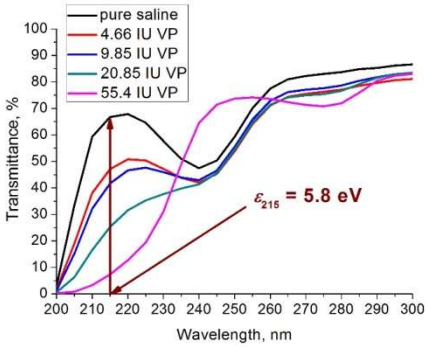

Figure 5. Dependence of the vasopressin solution transmittance on the concentration.

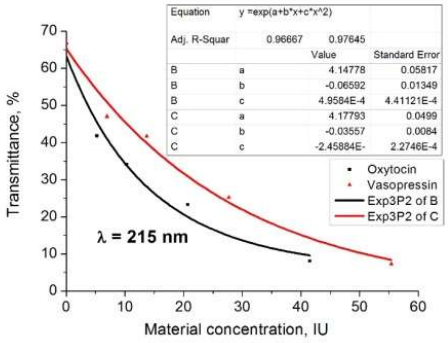

Figure 6. Neuropeptides transmittance dependence on the substance amount.

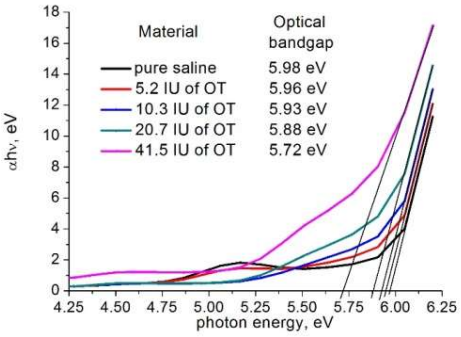

Figure 7. Optical absorption of the oxytocin solution characterization.

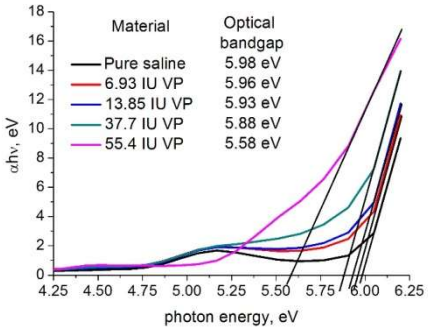

Figure 8. Optical absorption of the vasopressin solution characterization.

All recorded transmittance characteristics have strong absorption in the UV range. Therefore, we can define an optical bandgap for measured samples similar to those in semiconductor materials. For estimating the optical bandgap in the strong absorption region from the spectral dependence of the absorption coefficient, we apply the following equation [31,32]:

$$\alpha h\nu = B(h\nu - Eg)^n \qquad (1)$$

where, v is the photon frequency, h is the Plank's constant, B is an independent coefficient, Eg is the optical bandgap value, and the exponent n = 1 for our solutions, since the materials we measure are not semiconductors. Usually, for semiconductors, this exponent value is defined as n = 2 for semiconductors with indirect transition and n = 0.5 for semiconductors with direct transition. As shown by L. Joly et. al [30], the electron detachment under high-energy photon absorption is a two-stage process, therefore a peptide solution may be assigned as an indirect semiconductor, however, this question requires additional consideration. Graphical method for estimation of the optical bandgaps is shown in figures 7 and 8.

The linear behavior of the characteristics confirms the allowed indirect transition between valence and conductive bands in the measured solution. The extrapolation of the straight-lines to intercept with the energy axis gives the Eg values. The evaluation results demonstrate the evident dependence of optical bandgap on the neuropeptide amount in the solution. Optical bandgap of the pure saline is maximal and decreases with growth of the neuropeptide amount in the solution. Both studied neuropeptides behave in a similar way, however the calculated value of the optical bandgap is distinct for each substance.

According to the energy conservation law,

$$I_0 = I_R + I_T + I_A \quad (2)$$

where I_0, I_R, I_T and I_A are the intensities of incoming, reflected, transmitted and absorbed light, respectively. Therefore, reflectance of the studied neuropeptides we calculated using experimental data recorded via spectrometer. Figure 9 represents the calculated reflectance characteristics of the OT samples.

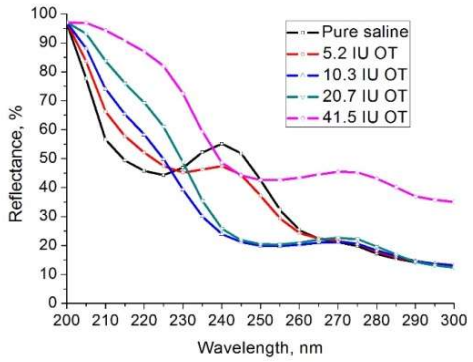

Figure 9. Dependence of the oxytocin solution reflectance on the concentration.

As can be seen, reflectance of the solution depends on the amount of the substance dissolved in saline. Reflectance rises with the increase of the OT amount. The AVP solutions demonstrate the same behavior. Figure 10 presents the calculated reflectance points for both studied neuropeptides. As in figure 6, we can see two sets of the experimental points and two fitting curves built using the same approximation model. Thus the difference between the two characteristics is evident. The reflectance data may therefore be used for differentiation of one peptide from another and for evaluation of the neuropeptide amounts in bodily fluids, for example in saliva [33].

It should be noted that our measurements were provided for the solutions with only one neuropeptide in the cuvette. Evaluation of the solutions with both or several substances simultaneous presenting in the same cuvette is the subject for a separate future study.

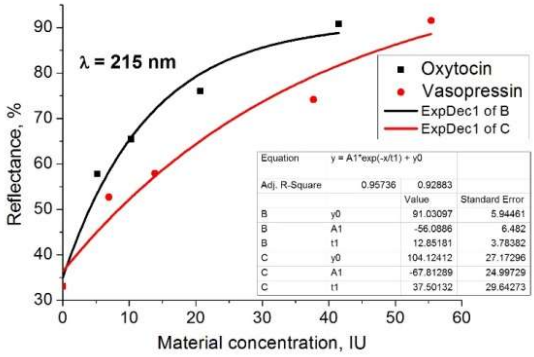

Figure 10. Neuropeptides reflectance dependence on the substance amount.

The additional important issue in neuropeptide assessment is the time during which the substances keep their properties and states. As known, OT is subject to degradation via deamidation, oxidation or thiol exchange [34]. Instability of OT in aqueous solutions has been noted in several sources [35,36]. However, the information presented by different authors is contradictory: for example, in-vitro pharmacologic studies show the half-life of oxytocin in the blood equal to 3-4 min [37]. Conversely, more recent researches claim OT stability during 30-40 or even 60 min [38]. We attempted to evaluate the stability time of OT and AVP using the optical bandgap of the studied substances.

The optical bandgap of a fluid solution depends on the material composition. Thus, one can assume that the optical bandgap change relates with the timed changes in the studied material. We calculated the optical bandgap of the solutions with the same neuropeptide concentration by transmittance and absorbance data, recorded through 3 hours with intervals of approximately 20 min. Figure 11 represents the calculated time-dependent characteristics.

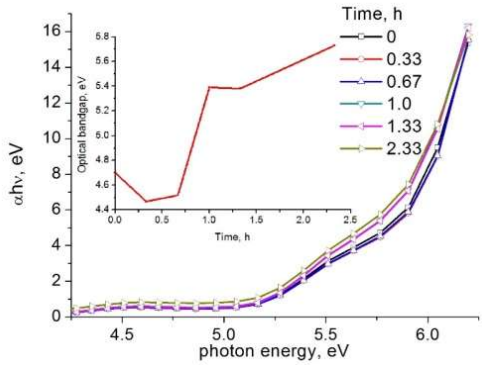

Figure 11. Lifetime of the oxytocin behavior.

Calculation of the measured characteristics shows that the optical bandgap rises with time. This dynamic indicates significant changes in the OT structure, its decomposition, as a result of which the bandgap increases and drifts to the value of pure saline. Thus, the oxytocin keeps its optical properties approximately 40-45 min, in agreement with other researchers [38]. Beyond that period, the optical bandgap begins to linearly increase, approaching the value of the pure saline. The same scenario was

observed for the vasopressin solutions, which kept their properties for approximately one hour.

4. CONCLUSIONS

In this work, we experimentally investigated the possibility to measure optical properties of the neuropeptides oxytocin and vasopressin, dissolved in saline. Results obtained from our experiments may be summarized as follows:

Optical measurements in the IR range are non-informative due to full light absorption by the saline itself.

Obtained results indicate that the most representative spectral region for correct measurement of OT and AVP is the mid-ultraviolet spectral area. These promising results enable assessment of these neuropeptides level in various solutions as well as differentiating oxytocin from vasopressin.

An optical bandgap of these peptide solutions may be used as a quality and quantity evaluation parameter, enabling differentiation of the peptides and evaluation of decomposition time.

Optical signals reflected from the sample with solutions of the two peptides bring enough information for evaluation.

Results of this study imply that optical methods of OT and AVP assay may provide a reliable and more accessible alternative to chemical assay kits. Refinement of the optical methods and technology used in this study may lead to development of less expensive, reusable and more user-friendly tools for these, and possibly other neuropeptides assessment. Further research and refinement is required, however, to develop and tune the technique and apparatus.

5. REFERENCES

[1] Chou C. L., Di Giovanni S. R., Mejia R., Nielsen S., Knepper M. A., Oxytocin as an antidiuretic hormone. I. Concentration dependence of action, *Am J Physiol-Renal*, 269, 1, F70-F77, **1995.**

[2] Landgraf R., Neumann I. D., Vasopressin and oxytocin release within the brain: a dynamic concept of multiple and variable modes of neuropeptide communication, *Front neuroendocrine.,* 25, 3, 150-176, **2004.**

[3] Schorscher-Petcu A., Dupré A., Tribollet E., Distribution of vasopressin and oxytocin binding sites in the brain and upper spinal cord of the common marmoset, *Neurosci let*., 461, 3, 217-222, **2009.**

[4] Campbell P,, Ophir A. G, Phelps S. M., Central vasopressin and oxytocin receptor distributions in two species of singing mice, *J Comp Neurol*., 516, 4*,* 321-333, **2009.**

[5] Benarroch E. E., Oxytocin and vasopressin Social neuropeptides with complex neuromodulatory functions, *Neurology*, 80, 16, 1521-1528, **2013.**

[6] Miller B. H, Takahashi J. S., Central circadian control of female reproductive function, *Front Endocrinol.,* 195, 4, 1-8, **2013.**

[7] Gibbs R., *Danforth's obstetrics and gynecology,* 10th ed. Philadelphia: Lippincott Williams & Wilkins, **2008.**

[8] Gutkowska J., Jankowski M., Antunes-Rodrigues J., The role of oxytocin in cardiovascular regulation, *Braz J Med Biol Res*, 47, 3, 206-214, **2014.**

[9] Mazzuca M., Minlebaev M., Shakirzyanova A. V., Tyzio R., Taccola G., Janackova S., Gataullina S., Ben-Ari Y., Giniatullin R. A., Khazipov R., Newborn analgesia mediated by oxytocin during delivery, *Front Cell Neurosci,* **2011.**

[10] Poutahidis T., Kearney S. M., Levkovich T., Qi P., Varian B. J., Lakritz J. R., Ibrahim Y. M., Chatzigiagkos A., Alm E.J., Erdman S.E., Microbial Symbionts Accelerate Wound Healing via the Neuropeptide Hormone Oxytocin. *PLOS one,* 8, 10, e78898, **2013.**

[11] Olff M., Frijling J. L., Kubzansky L. D., Bradley B., Ellenbogen M. A., Cardoso C., Bartz J. A., Yee J. R., van Zuiden M., The role of oxytocin in social bonding, stress regulation and mental health: An update on the moderating effects of context and interindividual differences, *Psychoneuroendocrino,* 38, 9, 1883-1894, **2013.**

[12] Feifel D., Macdonald K., Nguyen A., Cobb P., Warlan H., Galangue B., Minassian A., Becker O., Cooper J., Perry W., Lefebvre M., Adjunctive intranasal oxytocin reduces symptoms in schizophrenia patients, *Biological psychiatry*, 68, 7, 678-680, **2010.**

[13] Heinrichs M., Baumgartner T., Kirschbaum C., Ehlert U., Social support and oxytocin interact to suppress cortisol and subjective responses to psychosocial stress, *Biol psychiat*, 54, 12, 1389-1398, **2003.**

[14] Anagnostou E., Soorya L., Chaplin W., Bartz J., Halpern D., Wasserman S., Wang A.T., Pepa L., Tanel N., Kushki A., Hollander E,. Intranasal oxytocin versus placebo in the treatment of adults with autism spectrum disorders: a randomized controlled trial, *Mol Autism*, **2012.**

[15] Gordon I., Vander Wyk B. C., Bennett R. H., Cordeaux C., Lucas M. V., Eilbott J. A., Zagoory-Sharon O., Leckman J. F., Feldman R., Pelphrey K. A., Oxytocin enhances brain function in children with autism, *P Natl Acad Sci USA*, **2013.**

[16] Knepper M. A., Kwon T. H., Nielsen S., Molecular physiology of water balance, *New Engl J Med*, 372, 14, 1349-1358, **2015.**

[17] Albers H. E., The regulation of social recognition, social communication and aggression: vasopressin in the social behavior neural network, *Horm Behav*, 61, 3, 283-292, **2012.**

[18] Zink C. F., Stein J. L., Kempf L., Hakimi S., Meyer-Lindenberg A., Vasopressin modulates medial prefrontal cortex–amygdala circuitry during emotion processing in humans*, J Neurosci*, 30, 20, 7017-7022, **2010.**

[19] Carson D. S., Garner J. P., Hyde S. A., Libove R. A., Berquist S. W., Hornbeak K. B. Jackson L. P., Sumiyoshi R. D., Howerton C. L., Hannah S. L., Partap S., Arginine vasopressin is a blood-based biomarker of social functioning in children with autism, *PlOS one*, 10, 7, e0132224, **2015.**

[20] Griebel G., Simiand J., Stemmelin J., Gal C., Steinberg R., The vasopressin V1b receptor as a therapeutic target in stress-related disorders, *Curr Drug Targets CNS Neurol Disord*, 2, 3, 191-200, **2003.**

[21] Carter C. S., Sex differences in oxytocin and vasopressin: implications for autism spectrum disorders, *Behav brain res*, 176, 1, 170-186, **2007.**

[22] Meyer-Lindenberg A., Domes G., Kirsch P., Heinrichs M., Oxytocin and vasopressin in the human brain: social neuropeptides for translational medicine, *Nat Rev Neurosci*, 12, 9, 524-538, **2011.**

[23] Gruber C. W., Physiology of invertebrate oxytocin and vasopressin neuropeptides. *Exp Physiol*, 99, 1, 55-61, **2014.**

[24] Carter C. S., Pournajafi-Nazarloo H., Kramer K. M., Ziegler T. E. White-Traut R., Bello D., Schwertz D., Oxytocin: behavioral associations and potential as a salivary biomarker,. *Ann NY Acad Sci*, 1098, 312-322, **2007.**

[25] Grewen K. M., Davenport R. E., Light K. C., An investigation of plasma and salivary oxytocin responses in breast nand formula feeding mothers of infants, *Psychophysiology*, 47, 4, 625-632, **2010.**

[26] Weisman O., Schneiderman I., Zagoory-Sharon O., Feldman R., Salivary vasopressin increases following intranasal oxytocin administration, *Peptides*, 40, 99-103, **2013.**

[27] Szeto A., McCabe P. M., Nation D. A., Tabak B. A., Rossetti M. A., McCullough M. E., Schneiderman N., Mendez A. J,. Evaluation of enzyme immunoassay and radioimmunoassay methods for the measurement of plasma oxytocin, *Psychosom med*, 73, 5, 393, **2011.**

[28] McCullough M. E., Churchland P. S. Mendez A. J., Problems with measuring peripheral oxytocin: Can the data on oxytocin and human behavior be trusted, *Neurosci Biobehav Res*, 37, 8, 1485-1492, **2013.**

[29] Young S. N., Anderson G. M., Bioanalytical inaccuracy: a threat to the integrity and efficiency of research, *J Psychiatry Neurosci*, 35, 1, 3-6, **2010.**

[30] Joly L., Antoine R., Broyer M., Lemoine J., Dugourd P., Electron Photodetachment from Gas Phase Peptide Dianions. Relation with Optical Absorption Properties, *J Phys Chem A*, 112, 898-903, **2008.**

[31] Gnaser H., Energy and angular distribution of sputtered species, *Top. Appl. Phys*., 110, 231-238, **2007.**
[32] Axelevitch A., Eidin V., Gorenstein B., Effect of Photothermal Treatment on Properties of Ge Thin Films, *Journal of Material Science and Engineering A*, 3, 7, 475-480, **2013.**
[33] Axelevitch A., Akselevich V., Evaluation of the neuropeptides level using a surface enhanced mobile spectroscopy system, *Proceeding of IEEE Int'l. Conf. on Multisensor Fusion and Integration for Intelligent Systems (MFI-2015)*, San Diego, CA, USA, 334-338, **2015.**
[34] Hawe A., Poole R., Romeijn S., Kasper P., van der Heijden R., Jiskoot W., Towards Heat-stable Oxytocin Formulations: Analysis of Degradation Kinetics and Identification of Degradation Products, *Pharm Res*, 2, 7, 1679-1688, **2009.**
[35] Hogerzeil H. V., Walker G. J. A., De Goeje M. J., Stability of injectable oxytocin in tropical climates, *World Health Organization, Geneva*, WHO/DAP/93.6, **1993.**
[36] Trissel L. A., Zhang Y., Douglass K., Kastango E., Extended Stability 425 of oxytocin in common infusion solution, *Int J Pharm Compd*, 10, 156-158, **2006.**
[37] Rydén G., Sjöholm I., Half-life of oxytocin in blood of pregnant and non-pregnant women, *Acta Endocrinol (Copenh),* 61, 425-431, **1969.**
[38] Gonser M., Labor induction and augmentation with oxytocin: pharmacokinetic considerations, *Arch Gynecol Obstet*, 256, 63-66, **1995.**