Neurobiosensors and their possibilities of information signals coding

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Abstract. This publication is devoted to brief description of biotechnical devices - neuro-like biosensors as elements of biomedical information systems, analysis of electrical information signals at the output of biosensor, biosensor connections with electronic information systems, analysis of phenomenon of input information encoding by biosensor and possibilities of information protection in such information systems. The general definition of biosensors was given as well as prototypes and physical model. The neuro-like biosensor is considered as abstraction in consistent unity of its functions: signal receiver - filter - analyzer - encoder / decoder. A brief description of biosensor phenomenon and processes in it, as well as its functions were given. The possibilities of information coding by neurobiosensor within the following model were considered - at its input the information arrived being coded in the form of chemical structures of active substances or in the form of electric signals with the set characteristics, after the transformation (re-coding) the information appeared in the form of electric signals with the changed characteristics. It was shown that the reverse phenomenon - decoding of information is possible too. The work done demonstrated that the functions of neurobiosensor for encoding / decoding can be practically realized in two ways: in tabular form and in analytical form as function (or system of few functions). The example of practical application of neurobiosensor for encoding information about the structure of chemical substances in corresponding electric currents was given.

1. Introduction

It is well known that acceptation of new technologies always cause intensive progress in the areas of their application. This is true also for biosensors invention and their use in technique and technologies [1]; they are supposed also to be used for information protection. Indeed, methods of data protection using hardware electronic keys are well known. The usage of biotechnical devices – biosensors, incorporated into the electronic information systems allows realizing information protection in other ways; some of them may be suitable for the use in aerospace practice. Sometimes the confidentiality of the data in information systems with biosensors will be ensured much better than in traditionally used means and methods. Biosensors by themselves demonstrate memory properties and coding abilities, so, they potentially may be used for information encoding.

2. Biosensors as potential devices for information signals coding

In recent years, the problem of creating of electronic information systems (IS) with biosensors has become increasingly important. Biosensors (BS) are the elements of biological origin or artificial analogs of natural elements. In our previous publications on biomedical ISs [1], the authors had already raised the problem of high quality of biological and medical input data for such ISs [1]. A number of requirements for such input data for biomedical ISs were listed in some previous publications [1, 2]. This publication is devoted to brief description of biosensor (neurobiosensor NBS) connected with ISs; to analyzing of phenomenon of input information encoding by BS and peculiarities of information protection in information systems with BS. These data are input to the IS from biosensors in the form of electrical signals. Basing on own registered data, the authors considers the biosensor as analytical device - bioinformation system, which includes a neuro-like element with its properties. These properties means that NBS realizes simultaneously 4 functions: acceptor (or receiver) of information signals, filter, bioanalyzer and encoder / decoder of these signals. An electronic subsystem controls the functions of such biosensor with incorporated biological fragment (BF) and, finally, all this complex output its electrical signals being coupled with electronic IS.

Such biosensor by itself receives information that is encoded a) in the sequences of chemicals molecular structures influenced on biological fragment (BF), b) in characteristics of input electrical signals which differ always in comparison with output signals. The neuro-like element in the biosensor is characterized by a set of electrophysiological properties recorded in biophysical experiments; this type is called "neurobiosensor". The data given below in the article are the results of real measurements in experiments on brain neurons performed by Dr. Klyuchko O.M. on the basis in O.O.Bogomoletz Institute of Physiology of the National Academy of Sciences of Ukraine, and after that these data were processed and analysed at the National Aviation University (Kyiv).

The purpose of this work is a detailed description of technical devices - neuro-like biosensors as elements of biomedical information systems, analysis of electrical information signals at the output of biosensor, description of the ability to encode information with biosensor and information protection in such system. More detailed information on the possibilities of applying the results of work performed to protect information is given below.

Methods of investigations. Methods of modelling were used (physical and program modelling) based on the results of neurobiosensor biophysical, electrophysiological investigations. For biosensor characteristics studies the methods of patch-clamp and transmembrane electric currents recording in voltage-clamp conditions on the brain cells (neurons) were applied (Figs. 1, 2).

Brief description of biosensor. The authors definition of "biosensor" corresponds to the work experience of biosensor (neurobiosensor) study in biophysical experiments described below. The author considers "***biosensor"*** **(BS)** as analytical technical device (simultaneously it can be a bioinformation system), which unites a neuro-like element with its characteristics and four functions: acceptor (receiver) of information signals, filter, bioanalyzer and encoder/decoder of such signals. Electronic subsystem may be seen as biosensor part; it obtains output electrical signals from inserted in it biological fragment (BF). Other definitions of biosensor may be seen in contemporary literature but they do not contradict to our, but sometimes complement it.

3. Neurobiosensor: some prototypes

Developing the concept of neurobiosensor (NBS), the author proceeded from the results of biophysical experimental research with the registration of electric currents (signals) in voltage-clamp mode (or patch-clamp) on the cell membranes of living organisms (brain neurons as well). On the examples of such objects further work has been done - the incorporation of the NBS biosensor as element, as subsystem to electronic information systems (IS). The results of this work are presented in this article, they are protected by the patents of Ukraine [8, 9]. Accordingly, the following foreign works served as prototypes for our developed biosensor NBS.

The first prototype was a biosensor [10] which comprises a substrate having a buried electronic sensing element and a substrate surface above the buried electronic sensing element; a structured top

layer covering the substrate surface, having a top surface above the substrate surface, and comprising at least one stimulation and/or sensing electrode and a channel for holding the biomolecule by means of suction through said channel arranged between the top surface and the substrate surface, the sensing electrode being electrically coupled to the electronic sensing element; wherein the top surface is provided for placing a biomolecule present in a sample solution thereupon, the sensing electrode is provided for sensing electrical variations in and presence of the biomolecule [3].

The disadvantages of this method are that such biosensor was formed by a set of artificial objects, but not natural ones, including laboratory-synthesized molecules. So, it does not reflect the real mechanisms of influence of chemicals on natural objects, including electrical sensory processes in the Nature. As a result, it will not be enough successful to use this method to record chemicals effects on the properties of living objects (including electric currents of biomedical object, as well as the action of harmful and toxic substances of natural and artificial origin). In the prototype [3], it is impossible to track rapid changes in the characteristics of electric currents in natural objects. The electrical signals by themselves are almost impossible to subdivide against the background of noise. So, this calls into a question the quality and efficiency of such system [3].

The second prototype, the most close to the authors invention, is the invention [4] that realized the method of registration of receptors modifiers (antagonists) in some steps: (I) a BF with receptor modifiers was fractionated by the use of a liquid-based method, usually capillary electrophoresis, (II) a fraction with receptors modifiers (antagonist or modulator) was applied to a biosensor which was activated by an appropriate receptor agonist and demonstrated a measurable response. Said activation of the biosensor being pulsed by delivery of the receptor agonist to the biosensor for short period of times, said periods being separated by other periods when no agonist is delivered to the biosensor, and (III) the change of the resulting response from BF was measured using patch clamp technique. It is further possible to re-sensitize the biosensor desensitized as above by use of pulsed super fusion of the biosensor. This invention also relates to an apparatus usable for practicing the above mentioned method [4].

The disadvantages of prototype device and method [4] are that the biological fragment BF before its use is pre-treated in an imperfect way, which leads to: 1- death of biological fragments, 2- in case of survival of biological fragments electrical signals from them cannot be registered well because of noise. As a result, accordingly, it is impossible to obtain an electrical signal of satisfactory quality at BF output, therefore, it will not be successful to use such a device and relative method for recording the effects of chemicals on electric currents of biomedical object (including harmful and toxic substances and artificial origin). This calls into a question the quality and efficiency of prototype system [4] and quality and reliability of measurements.

4. Biosensors with "biological fragments"

"Biological fragments" (BF) may be fragments of living organisms and they can act as biosensors [5-9]. Some of BF can be in chemical reactions with other chemicals and substances; due to this they can be BS sensitive (sensory) part. For the study of such objects the methods of electrophysiological, biophysical researches are used, for example, patch-clamp, transmembrane electric currents recording under the voltage-clamp conditions, others (Fig. 2). The numerical data obtained in the process of such experiments with brain cells (neurons) can be digitized and input further to electronic information systems; such data characterize the processes in living organisms. The complex of BF with experimental electrophysiological setup (EPS) and with information systems (ISs) can be seen as united information bioelectronic complex: BF – EPS – ISs (Fig. 1) [5]. The scheme of technical system of biosensor (neurobiosensor) linked with electronic information system and Internet used for electrophysiological study of electric transmembrane currents under the voltage-clamp conditions are on Figures 1, 2. Invention of experimental methods was based on methods previously developed by biophysical research groups under the leadership of Academicians of the USSR Academy of Sciences and the National Academy of Sciences of Ukraine, Profs. Kostyuk P. G. and Kryshtal O. A. where the author Dr. Klyuchko O. M. worked for a long time and used these

techniques for investigations. The scheme of biosensor operation, including electronic measurements is suggested on Figure 2.

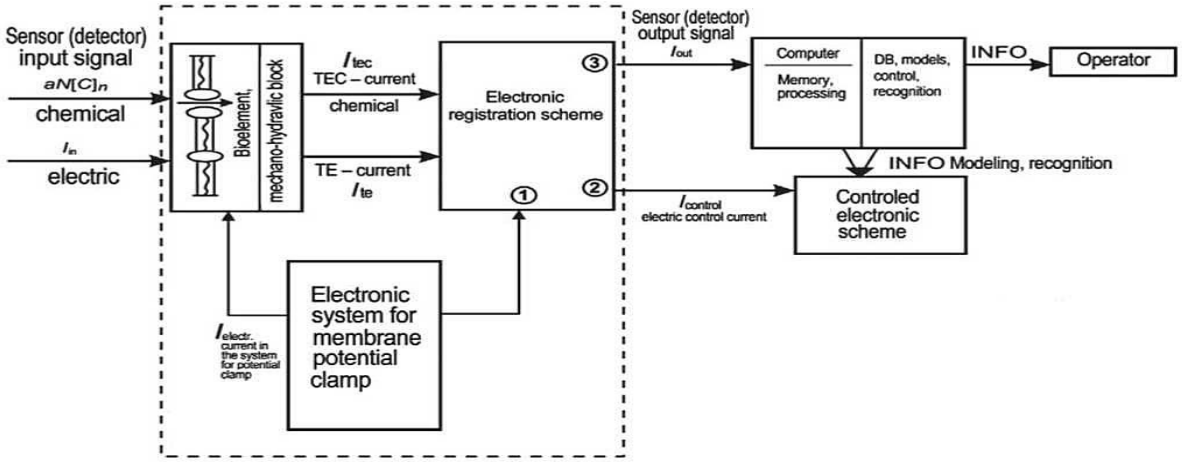

Figure 1. The scheme of technical system of biosensor (neurobiosensor) linked with electronic information system and Internet. Input information (as electrical or chemical signals) was obtained primarily by biosensor system (drawn on the left side of the scheme). Encoded information was transmitted at biosensor output as electrical signals with changed characteristics.

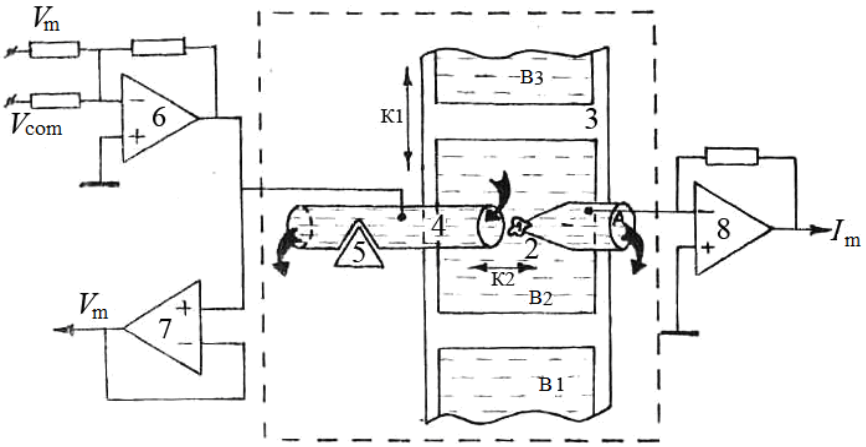

Figure 2. The scheme of mechanical, hydrostatic and electrical parts of experimental setup used for the registration of transmembrane electrical currents under the voltage-clamp conditions. *Significations.* 1 – Excitable cell (neuron) at the top of micropipette. 2 - Micropipette filled with intracellular solution; 3 - Mobile cassette with experimental chambers with different solutions for the application (B1, B2, B3); arrow K1 shows the directions of movement of these chambers with solutions. 4 – Preserving tube for the cell movement from one chamber to another. After the opening of electromagnetic valve 5, a quick application of B2 solution have happened (the solution was sucked into the tube in the result of the jump of a negative hydrostatic pressure). The black arrows indicate the directions of solutions flows during experiment. The dotted line shows the mechanical and hydrostatical parts of experimental complex. 6 - Amplifier for Vm the holding transmembrane potential and command Vcom. 7 - Unit for potential measurement. 8 - Amplifier for registered transmembrane currents [8].

5. Abstract imagination about NBS four main functions

Four NBS main functions were studied profoundly by many scientific groups and authors in XX century. Considering NBS as abstract issue, one can list these four NBS functions. They co-exceed, respectively, with four NBS main structural elements (Figure 3).

1) NBS – receiver (acceptor) of information signals (Figure 3). The input of NBS information is going in two ways, in the form of signals of two types – chemical and electrical signals.

1. Input of NBS information encoded in chemicals structures influencing on BF membrane receptors. In this case 1, characteristics of output electrical signals correspond to chemicals structures which influence on BF. So, input chemical signals were encoded into electrical ones.

2. Input information is received by NBS as electrical signals with defined characteristics, and at NBS output these electrical signals have characteristics different from input ones. So, electrical signals with defined characteristics are transformed (encoded) to electrical signals with other characteristics.

2) NBS – filter of input electrical information signals (Figure 3). NBS input signals "filtering" is going because they must interact *only with peculiar* BF membrane molecular structures.

Described functions 1) and 2) determine the NBS role as *key code* in information accepting and transmission (Figure 3).

3) NBS – primary elementary analyzer of input information signals (Figure 3). Molecular structure of NBS membrane in complex with some other inner membrane phenomena determine the NBS function as "analyzer" of input information signals. So, the number of NBS properties and phenomena 1), 2), 3) causes that biosensor "distinguishing" the chemicals that interact with it [5-9]. On this base Klyuchko O.M. proposed few new methods of qualitative and quantitative analysis of chemicals and four patents of Ukraine protect priority of these invented methods [5-9].

4) NBS – information encoder/decoder (Figure 3). During XX c. it was demonstrated in numerous experiments that NBS receives information as information signals – chemicals of defined structures, or electrical signals with certain characteristics (that can be recoded into electrical signals with another characteristics at NBS level). This process can going in opposite direction too: decoding is possible as it was shown in different numerous experiments (Figure 3). It can be proved that the functions of NBS biosensor for encoding/decoding can be expressed in: 1) tabular form, and 2) analytical form as a function or system of functions. The example of experimentally registered coding of chemical signal (substance AR) into electrical ones is represented on Figure 4.

6. Examples and possible ways of information protection basing on the methods and achievements of biophysics

The above described techniques and results allow implementing data protection in information systems using these phenomena and principles.

1. *BF as "key-lock".* The principle of "key-lock" is extremely common in the Nature, and can be used for information protection in technical devices, for example, for information protection in some segments of information network. When making device with BF from specific types of artificial synthetic analogues - BF molecules in the information network, it is possible to "disconnect" specifically certain segment of BF devices, preventing data outflow. The "key" for "connection" or "disconnection" (or to the balance between confidentiality and availability of information in the system) are devices that contain BF with specific molecular composition.

2. *Confidentiality of information inbioinformation systems with BSs.* Increasing the level of information confidentiality using devices with BF can be realized by assembling specifically selected types of molecules, changing them or their positions in space, and etc. So, because an information that is "passed" depends directly on the types of molecules in devices with BF, it is possible to control this information to some extent.

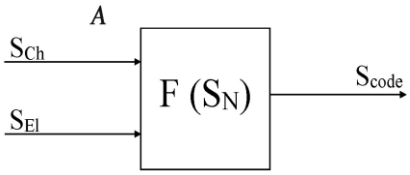

Figure 3. Representation of neurobiosensor function as encoder of input information signals. *Significations*: F - coding function, S_N - signals of N quantity as arguments of function of coding, S_{Ch} – input information signals encoded in molecular structures of chemicals, S_{El} – input electrical information signals.

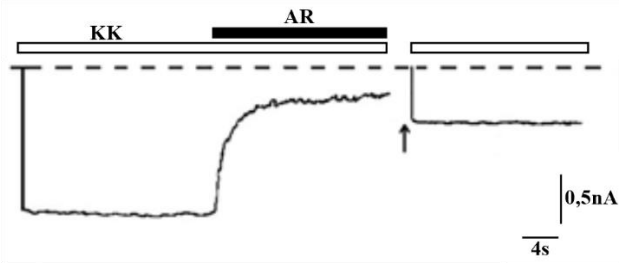

Figure 4. Example of biosensor functioning: information signals from biosensor registered during electrophysiological experiment. At the input of biosensor was the substance kainate (KK), which initiated steady transmembrane electric currents. After some time against the background of the current the substance argiopin (AR), which reduced the current amplitude was added. At the output of biosensor stationary electric currents with smaller amplitude were registered (recording on the right after the arrow). The action of AR was characterized by the number of electrical characteristics, specific for the used substance (kinetic constants and rates of currents amplitudes decrease, other numerical values). On the BF of biosensor holding potential was -100 mV.

Conclusions

In present article the general definition and description of biosensors (neurobiosensors - NBS) were suggested as well as prototypes and physical model. The concept of neuronlike biosensor NBS was created on the base of results of biophysical investigations of the neurons from mammalian brain (hippocampus). Such neurobiosensor according to its functions may be imagined in unity of its four functions: signal receiver - filter - analyzer - encoder / decoder. A brief analysis of all NBS functions were done.

The natural NBS ability of information coding was analyzed deeply. At NBS input the information is received being coded in the sequences of chemical units (atoms or molecules), or in electric signals with input characteristics, after the re-coding in NBS the information is obtained in electric signals with transformed characteristics. It is known that reverse phenomenon - information decoding by NBS is possible too. The possibilities of NBS use for information signals coding were observed; they opens new prospects for information protection in biotechnical information systems. The work done demonstrated that the functions of NBS for encoding / decoding of chemicals structures can be practically realized in two ways: in tabular form and in analytical form as a function (or a system of few functions). There are about few thousand of such “NBS-active” chemicals with known structures were studied for today as well as regularities of their interaction with NBS [10-27]. Being organized into the databases, these chemical substances and regularities of their interactions with NBS can be used for automatic encoding with further transmission of relevant information about chemical

substances. Elaborated techniques and devices described in present article are defended by the patents of Ukraine [8, 9]. The phenomena of information encoding by BS (NBS) and its application in technique are important indeed. As it was demonstrated in present article, the natural biosensor NBS can fulfill the functions of devices, which in technique were called encoders/decoders of information.

References

[1] Klyuchko O M 2008 Information and computer technologies in biology and medicine. (Kyiv: NAU-druk) 252 p. [In Ukrainian].

[2] Klyuchko O M and Klyuchko Z F 2018 Electronic databases for Arthropods: methods and applications. *Biotechnol. acta.* **11**(4):28–49. https://doi.org/10.15407/biotech11.04.028.

[3] Xian Tong Chen, Min Bin Yu, Han Hua Feng and Guo Qiang Lo Biosensor. Patent WO 2007084076 A1 / Agency For Science, Technology And Research. Priority: 20.6.2006. Issued: 26.8.2007. http://www.google.com/patents/WO2007084076A1?hl=en&cl=en.

[4] Owe Orwar and Jardemark Kent Biosensors and methods of using the same. Patent US 20020182642 A1, DE69832381D1. Priority: 19.6.2002. Issued: 5.12. 2002. https://www.google.com/patents/US20020182642.

[5] Klyuchko O M 2020 Venom and toxins from Argiope lobata: electrophysiological studies. *Biol. Stud.***14**(2):39-56.

[6] Sigvors F, Sackman B, Sigvors F and Neer E 1987 Registration of single channels. M.: Mir, 1987. - 448 p. [In Russian].

[7] Klyuchko O M and Biletsky A Ya 2019 Computer recognition of chemical substances based on their electrophysiological characteristics. *Biotechnol. acta*.**12**(5):5-28. https://doi.org/10.15407/biotech12.05.005.

[8] Klyuchko O M, Biletsky A Ya and Navrotsky D O Method of application of biosensor test system. Patent UA 129923 U, IPC G01N33 / 00, G01N33 / 50, C12Q 1/02. Priority: 22.03.2018, u201802896. Issued: 26.11.2018, Bull. 22. [In Ukrainian].

[9] Klyuchko O M, Biletsky A Ya and Navrotsky D O Method of application of biotechnical monitoring system with biosensor (biosensor test system). Patent UA 132245 U, G01N33 / 50. Priority: 23.03.18, u2018 02893. Issued: 25.02.2019, Bull. 4. [In Ukrainian].

[10] Aramaki Y, Yashuhara T, Higashijima T, Yoshioka M, Miwa A, Kawai N and Nakajima T 1986 Chemical characterization of spider toxins JSTX and NSTX. *Proc. JapanAcademy.*:**62**(9):1012-1014. [DOI: https://doi.org/10.2183/pjab.62.359; Google Scholar]

[11] Casewell N R, Wüster W, Vonk F J, Harrison R A and Fry B G2013 Complex cocktails: the evolutionary novelty of venoms. *Trends EcolEvol.***28**(4):219-29. [DOI: https://doi.org/10.1016/j.tree.2012.10.020; PMed:23219381; Google Scholar]

[12] Cavigliasso F, Mathé-Hubert H, Kremmer L, Rebuf C., Gatti J L, Malausa T, Colinet D and Poirié M. 2019 Rapid and Differential Evolution of the Venom Composition of a Parasitoid Wasp Depending on the Host Strain. *Toxins (Basel).*:**11**(11):629-647. [DOI: https://doi.org/10.3390/toxins11110629; PMid:31671900 ; Google Scholar]

[13] Cordell G A and Brossi A 1994 Chemistry and Pharmacology. The Alkaloids. USA:*Academic Press.*:280 p.

[14] Daly N L and Wilson D 2018 Structural diversity of arthropod venom toxins. *Toxicon.*:152:46-56. doi: 10.1016/j.toxicon.2018.07.018. [DOI: https://doi.org/10.1016/j.toxicon.2018.07.018; PMid:30040988; Google Scholar]

[15] Herz W, Kirby G W, Moore R E, Steglich W and Tamm Ch 2012 Fortschritte der Chemie organischer Naturstoffe. In: Progress in the Chemistry of Organic Natural Products. USA:*Springer Science & Business Media*.66:332 p.

[16] Grishin E 2016 Spider toxins active on purinergic P2X3 receptor. *Toxicon.*:116:72. [DOI: https://doi.org/10.1016/j.toxicon.2016.01.003; Google Scholar]
[17] Herzig V 2019 Arthropod assassins: Crawling biochemists with diverse toxin pharmacopeias. *Toxicon.*:158:33-37. [DOI: https://doi.org/10.1016/j.toxicon.2018.11.312; Google Scholar]
[18] Jackson H, Usherwood F N R 1988 Spider toxins as tools for dissecting elements of excitatory amino acids transmission. *Trends In Neurosci.*:**11**(6):278-283. [DOI: https://doi.org/10.1016/0166-2236(88)90112-9; Google Scholar]
[19] Jankovic J, Albanese A, Atassi M Z, Dolly J O, Hallett M and Mayer N H 2009 Botulinum Toxin E-Book: Therapeutic Clinical Practice and Science. USA: *Elsevier Health Sciences*.:512 p.
[20] Kachel H S, Buckingham S D and Sattelle D B 2018 Insect toxins - selective pharmacological tools and drug/chemical leads. *CurrOpin Insect Sci*.:30:93-98. [DOI: https://doi.Org/10.1016/j.cois.2018.10.001; Google Scholar]
[21] Kusano Tomonobu and Suzuki Hideyuki 2015 Polyamines: A Universal Molecular Nexus for Growth, Survival, and Specialized Metabolism. USA: *Springer*.:336 p. [DOI: https://doi.org/10.1007/978-4-431-55212-3; Google Scholar]
[22] Lajoiea M, Zobel-Thropp B A, Delahaye B, Roberts S, Kumirov V K and Bandarian V, Binford G J, Cordesa M H J 2016 The chemistry and functional diversity of spider phospholipase D toxins.*Toxicon*.:116:79. [DOI: https://doi.org/10.1016/j.toxicon.2016.01.025 Google Scholar]
[23] Senji Laxme R R, Suranse V and Sunagar K 2019Arthropod venoms: Biochemistry, ecology and evolution. *Toxicon.*:158:84-103. [DOI: https://doi.org/10.1016/j.toxicon.2018.11.433; Google Scholar]
[24] Schwartz E F, Mourão C B, Moreira K G, Camargos T S and Mortari M R 2012 Arthropod venoms: a vast arsenal of insecticidal neuropeptides. Biopolymers.:**98**(4):385-405. [DOI: https://doi.org/10.1002/bip.22100; Google Scholar]
[25] Walker A A, Robinson S D, Yeates D K, Jin J, Baumann K, Dobson J, Fry B G and King G F 2018 Entomo-venomics: The evolution, biology and biochemistry of insect venoms. *Toxicon.*:154:15-2. [DOI: https://doi.org/10.1016/j.toxicon.2018.09.004; Google Scholar]
[26] Walker A A, Rosenthal M, Undheim E E A and King G F 2018 Harvesting Venom Toxins from Assassin Bugs and Other Heteropteran Insects. *J VisExp.*:(134). [DOI: https://doi.org/10.3791/57729; Google Scholar]
[27] Zlotkin E 1985Toxins derived from Arthropodvenoms specially affecting insects. In: Kerkut GA, GilbertLI (Ed.) *Compre hensive Insect Physiology, Biochemistry and Pharmacology.* (Oxford: Pergamon Press).:10:499-546.