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ЕЖЕМЕСЯЧНЫЙ НАУЧНЫЙ ЖУРНАЛ

Медицинские новости Грузии

საქართველოს სამედიცინო სიახლენი

GEORGIAN MEDICAL NEWS

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GMN: Georgian Medical News is peer-reviewed, published monthly journal committed to promoting the science and art of medicine and the betterment of public health, published by the GMN Editorial Board since 1994. GMN carries original scientific articles on medicine, biology and pharmacy, which are of experimental, theoretical and practical character; publishes original research, reviews, commentaries, editorials, essays, medical news, and correspondence in English and Russian.

GMN is indexed in MEDLINE, SCOPUS, PubMed and VINITI Russian Academy of Sciences. The full text content is available through EBSCO databases.

GMN: Медицинские новости Грузии - ежемесячный рецензируемый научный журнал, издаётся Редакционной коллегией с 1994 года на русском и английском языках в целях поддержки медицинской науки и улучшения здравоохранения. В журнале публикуются оригинальные научные статьи в области медицины, биологии и фармации, статьи обзорного характера, научные сообщения, новости медицины и здравоохранения. Журнал индексируется в MEDLINE, отражён в базе данных SCOPUS, PubMed и ВИНИТИ РАН. Полнотекстовые статьи журнала доступны через БД EBSCO.

GMN: Georgian Medical News – საქართველოს სამედიცინო სიახლენი – არის ყოველთვიური სამეცნიერო სამედიცინო რეცენზირებადი ჟურნალი, გამოიცემა 1994 წლიდან, წარმოადგენს სარედაქციო კოლეგიისა და აშშ-ის მეცნიერების, განათლების, ინდუსტრიის, ხელოვნებისა და ბუნებისმეტყველების საერთაშორისო აკადემიის ერთობლივ გამოცემას. GMN-ში რუსულ და ინგლისურ ენებზე ქვეყნდება ექსპერიმენტული, თეორიული და პრაქტიკული ხასიათის ორიგინალური სამეცნიერო სტატიები მედიცინის, ბიოლოგიისა და ფარმაციის სფეროში, მიმოხილვითი ხასიათის სტატიები.

ჟურნალი ინდექსირებულია MEDLINE-ის საერთაშორისო სისტემაში, ასახულია SCOPUS-ის, PubMed-ის და ВИНИТИ РАН-ის მონაცემთა ბაზებში. სტატიების სრული ტექსტი ხელმისაწვდომია EBSCO-ს მონაცემთა ბაზებიდან.

WEBSITE

www.geomednews.com

К СВЕДЕНИЮ АВТОРОВ!

При направлении статьи в редакцию необходимо соблюдать следующие правила:

1. Статья должна быть представлена в двух экземплярах, на русском или английском языках, напечатанная через **полтора интервала на одной стороне стандартного листа с шириной левого поля в три сантиметра**. Используемый компьютерный шрифт для текста на русском и английском языках - **Times New Roman** (**Кириллица**), для текста на грузинском языке следует использовать **AcadNusx.** Размер шрифта - **12**. К рукописи, напечатанной на компьютере, должен быть приложен CD со статьей.

2. Размер статьи должен быть не менее десяти и не более двадцати страниц машинописи, включая указатель литературы и резюме на английском, русском и грузинском языках.

3. В статье должны быть освещены актуальность данного материала, методы и результаты исследования и их обсуждение.

При представлении в печать научных экспериментальных работ авторы должны указывать вид и количество экспериментальных животных, применявшиеся методы обезболивания и усыпления (в ходе острых опытов).

4. К статье должны быть приложены краткое (на полстраницы) резюме на английском, русском и грузинском языках (включающее следующие разделы: цель исследования, материал и методы, результаты и заключение) и список ключевых слов (key words).

5. Таблицы необходимо представлять в печатной форме. Фотокопии не принимаются. **Все цифровые, итоговые и процентные данные в таблицах должны соответствовать таковым в тексте статьи**. Таблицы и графики должны быть озаглавлены.

6. Фотографии должны быть контрастными, фотокопии с рентгенограмм - в позитивном изображении. Рисунки, чертежи и диаграммы следует озаглавить, пронумеровать и вставить в соответствующее место текста **в tiff формате**.

В подписях к микрофотографиям следует указывать степень увеличения через окуляр или объектив и метод окраски или импрегнации срезов.

7. Фамилии отечественных авторов приводятся в оригинальной транскрипции.

8. При оформлении и направлении статей в журнал МНГ просим авторов соблюдать правила, изложенные в «Единых требованиях к рукописям, представляемым в биомедицинские журналы», принятых Международным комитетом редакторов медицинских журналов - http://www.spinesurgery.ru/files/publish.pdf и http://www.nlm.nih.gov/bsd/uniform_requirements.html
В конце каждой оригинальной статьи приводится библиографический список. В список литературы включаются все материалы, на которые имеются ссылки в тексте. Список составляется в алфавитном порядке и нумеруется. Литературный источник приводится на языке оригинала. В списке литературы сначала приводятся работы, написанные знаками грузинского алфавита, затем кириллицей и латиницей. Ссылки на цитируемые работы в тексте статьи даются в квадратных скобках в виде номера, соответствующего номеру данной работы в списке литературы. Большинство цитированных источников должны быть за последние 5-7 лет.

9. Для получения права на публикацию статья должна иметь от руководителя работы или учреждения визу и сопроводительное отношение, написанные или напечатанные на бланке и заверенные подписью и печатью.

10. В конце статьи должны быть подписи всех авторов, полностью приведены их фамилии, имена и отчества, указаны служебный и домашний номера телефонов и адреса или иные координаты. Количество авторов (соавторов) не должно превышать пяти человек.

11. Редакция оставляет за собой право сокращать и исправлять статьи. Корректура авторам не высылается, вся работа и сверка проводится по авторскому оригиналу.

12. Недопустимо направление в редакцию работ, представленных к печати в иных издательствах или опубликованных в других изданиях.

При нарушении указанных правил статьи не рассматриваются.

REQUIREMENTS

Please note, materials submitted to the Editorial Office Staff are supposed to meet the following requirements:

1. Articles must be provided with a double copy, in English or Russian languages and typed or compu-ter-printed on a single side of standard typing paper, with the left margin of **3** centimeters width, and **1.5** spacing between the lines, typeface - **Times New Roman** (**Cyrillic**), print size - **12** (referring to Georgian and Russian materials). With computer-printed texts please enclose a CD carrying the same file titled with Latin symbols.

2. Size of the article, including index and resume in English, Russian and Georgian languages must be at least 10 pages and not exceed the limit of 20 pages of typed or computer-printed text.

3. Submitted material must include a coverage of a topical subject, research methods, results, and review.

Authors of the scientific-research works must indicate the number of experimental biological species drawn in, list the employed methods of anesthetization and soporific means used during acute tests.

4. Articles must have a short (half page) abstract in English, Russian and Georgian (including the following sections: aim of study, material and methods, results and conclusions) and a list of key words.

5. Tables must be presented in an original typed or computer-printed form, instead of a photocopied version. **Numbers, totals, percentile data on the tables must coincide with those in the texts of the articles.** Tables and graphs must be headed.

6. Photographs are required to be contrasted and must be submitted with doubles. Please number each photograph with a pencil on its back, indicate author's name, title of the article (short version), and mark out its top and bottom parts. Drawings must be accurate, drafts and diagrams drawn in Indian ink (or black ink). Photocopies of the X-ray photographs must be presented in a positive image in **tiff format**.

Accurately numbered subtitles for each illustration must be listed on a separate sheet of paper. In the subtitles for the microphotographs please indicate the ocular and objective lens magnification power, method of coloring or impregnation of the microscopic sections (preparations).

7. Please indicate last names, first and middle initials of the native authors, present names and initials of the foreign authors in the transcription of the original language, enclose in parenthesis corresponding number under which the author is listed in the reference materials.

8. Please follow guidance offered to authors by The International Committee of Medical Journal Editors guidance in its Uniform Requirements for Manuscripts Submitted to Biomedical Journals publication available online at: http://www.nlm.nih.gov/bsd/uniform_requirements.html
http://www.icmje.org/urm_full.pdf
In GMN style for each work cited in the text, a bibliographic reference is given, and this is located at the end of the article under the title "References". All references cited in the text must be listed. The list of references should be arranged alphabetically and then numbered. References are numbered in the text [numbers in square brackets] and in the reference list and numbers are repeated throughout the text as needed. The bibliographic description is given in the language of publication (citations in Georgian script are followed by Cyrillic and Latin).

9. To obtain the rights of publication articles must be accompanied by a visa from the project instructor or the establishment, where the work has been performed, and a reference letter, both written or typed on a special signed form, certified by a stamp or a seal.

10. Articles must be signed by all of the authors at the end, and they must be provided with a list of full names, office and home phone numbers and addresses or other non-office locations where the authors could be reached. The number of the authors (co-authors) must not exceed the limit of 5 people.

11. Editorial Staff reserves the rights to cut down in size and correct the articles. Proof-sheets are not sent out to the authors. The entire editorial and collation work is performed according to the author's original text.

12. Sending in the works that have already been assigned to the press by other Editorial Staffs or have been printed by other publishers is not permissible.

Articles that Fail to Meet the Aforementioned Requirements are not Assigned to be Reviewed.

ავტორთა საყურადღებოდ!

რედაქციაში სტატიის წარმოდგენისას საჭიროა დავიცვათ შემდეგი წესები:

1. სტატია უნდა წარმოადგინოთ 2 ცალად, რუსულ ან ინგლისურ ენებზე,დაბეჭდილი სტანდარტული ფურცლის 1 გვერდზე, 3 სმ სიგანის მარცხენა ველისა და სტრიქონებს შორის 1,5 ინტერვალის დაცვით. გამოყენებული კომპიუტერული შრიფტი რუსულ და ინგლისურენოვან ტექსტებში - **Times New Roman (Кириллица)**, ხოლო ქართულენოვან ტექსტში საჭიროა გამოვიყენოთ **AcadNusx.** შრიფტის ზომა – 12. სტატიას თან უნდა ახლდეს CD სტატიით.

2. სტატიის მოცულობა არ უნდა შეადგენდეს 10 გვერდზე ნაკლებს და 20 გვერდზე მეტს ლიტერატურის სიის და რეზიუმეების (ინგლისურ, რუსულ და ქართულ ენებზე) ჩათვლით.

3. სტატიაში საჭიროა გაშუქდეს: საკითხის აქტუალობა; კვლევის მიზანი; საკვლევი მასალა და გამოყენებული მეთოდები; მიღებული შედეგები და მათი განსჯა. ექსპერიმენტული ხასიათის სტატიების წარმოდგენისას ავტორებმა უნდა მიუთითონ საექსპერიმენტო ცხოველების სახეობა და რაოდენობა; გაუტკივარებისა და დაძინების მეთოდები (მწვავე ცდების პირობებში).

4. სტატიას თან უნდა ახლდეს რეზიუმე ინგლისურ, რუსულ და ქართულ ენებზე არანაკლებ ნახევარი გვერდის მოცულობისა (სათაურის, ავტორების, დაწესებულების მითითებით და უნდა შეიცავდეს შემდეგ განყოფილებებს: მიზანი, მასალა და მეთოდები, შედეგები და დასკვნები; ტექსტუალური ნაწილი არ უნდა იყოს 15 სტრიქონზე ნაკლები) და საკვანძო სიტყვების ჩამონათვალი (key words).

5. ცხრილები საჭიროა წარმოადგინოთ ნაბეჭდი სახით. ყველა ციფრული, შემაჯამებელი და პროცენტული მონაცემები უნდა შეესაბამებოდეს ტექსტში მოყვანილს.

6. ფოტოსურათები უნდა იყოს კონტრასტული; სურათები, ნახაზები, დიაგრამები - დასათაურებული, დანომრილი და სათანადო ადგილას ჩასმული. რენტგენოგრამების ფოტოასლები წარმოადგინეთ პოზიტიური გამოსახულებით **tiff** ფორმატში. მიკროფოტოსურათების წარწერებში საჭიროა მიუთითოთ ოკულარის ან ობიექტივის საშუალებით გადიდების ხარისხი, ანათალების შეღებვის ან იმპრეგნაციის მეთოდი და აღნიშნოთ სურათის ზედა და ქვედა ნაწილები.

7. სამამულო ავტორების გვარები სტატიაში აღინიშნება ინიციალების თანდართვით, უცხოურისა – უცხოური ტრანსკრიპციით.

8. სტატიას თან უნდა ახლდეს ავტორის მიერ გამოყენებული სამამულო და უცხოური შრომების ბიბლიოგრაფიული სია (ბოლო 5-8 წლის სიღრმით). ანბანური წყობით წარმოდგენილ ბიბლიოგრაფიულ სიაში მიუთითეთ ჯერ სამამულო, შემდეგ უცხოელი ავტორები (გვარი, ინიციალები, სტატიის სათაური, ჟურნალის დასახელება, გამოცემის ადგილი, წელი, ჟურნალის №, პირველი და ბოლო გვერდები). მონოგრაფიის შემთხვევაში მიუთითეთ გამოცემის წელი, ადგილი და გვერდების საერთო რაოდენობა. ტექსტში კვადრატულ ფჩხილებში უნდა მიუთითოთ ავტორის შესაბამისი N ლიტერატურის სიის მიხედვით. მიზანშეწონილია, რომ ციტირებული წყაროების უმეტესი ნაწილი იყოს 5-6 წლის სიღრმის.

9. სტატიას თან უნდა ახლდეს: ა) დაწესებულების ან სამეცნიერო ხელმძღვანელის წარდგინება, დამოწმებული ხელმოწერითა და ბეჭდით; ბ) დარგის სპეციალისტის დამოწმებული რეცენზია, რომელშიც მითითებული იქნება საკითხის აქტუალობა, მასალის საკმაობა, მეთოდის სანდოობა, შედეგების სამეცნიერო-პრაქტიკული მნიშვნელობა.

10. სტატიის ბოლოს საჭიროა ყველა ავტორის ხელმოწერა, რომელთა რაოდენობა არ უნდა აღემატებოდეს 5-ს.

11. რედაქცია იტოვებს უფლებას შეასწოროს სტატია. ტექსტზე მუშაობა და შეჯერება ხდება საავტორო ორიგინალის მიხედვით.

12. დაუშვებელია რედაქციაში ისეთი სტატიის წარდგენა, რომელიც დასაბეჭდად წარდგენილი იყო სხვა რედაქციაში ან გამოქვეყნებული იყო სხვა გამოცემებში.

აღნიშნული წესების დარღვევის შემთხვევაში სტატიები არ განიხილება.

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INTERACTION OF DOPAMINE WITH DNA, DEPENDING ON THE IONIC STRENGTH OF THE SOLUTION: POTENTIAL APPLICATION IN SENSOR TECHNOLOGY

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Abstract.

Introduction: Dopamine is one of the most important neurotransmitters in the central nervous system. Along with other mediators, such as serotonin, noradrenaline, adrenaline, it enormously contributes to the human mental health. Any concentration alterations of dopamine in the brain result in devastating consequences, expressed as mental disorders, movement deviations, etc. From this point of view, it is crucial to make possible to determine the concentration of dopamine in the blood, and, with more probability, in the urine. Dopamine concentration monitoring may be possible by implementation to create dopamine-sensitive biosensors, where there should be an underlayer, interacting with and detecting dopamine. Nucleic acids, particularly, DNA can serve as sensitive biomolecules for dopamine-sensors.

Methods: In this work, the interaction of DNA with dopamine, depending on the ionic strength of the solution, has been studied, using the method of UV-melting and absorption spectroscopy. In the experiments calf thymus DNA, dopamine hydrochloride ("Sigma-Aldrich", USA), physiological solution were used. Concentrations of DNA and dopamine were determined spectrophotometrically, using the following values of the extinction coefficients: ε_{260}=6600 $M^{-1}cm^{-1}$ for DNA and ε_{280}=2200 $M^{-1}cm^{-1}$ for dopamine hydrochloride. The experiments were carried out at the ionic strengths of the solution 0.02 M and 0.01 M Na^+. The medium pH was equal to 7.0.

Result: For this aim, the interaction between dopamine and DNA, depending on the solution ionic strength, was explored to reveal whether there exists a binding or not. The results, obtained in this study, show that dopamine binds to DNA, at least, in two regions at low ionic strength of the solution and, at least, in three regions at high ionic strength of the solution. It was shown that DNA-dopamine complex melting curve is shifted toward high temperatures, as compared to that of DNA. It results in melting temperature increasing by more, than 3-4°C, in high concentration ratios dopamine/DNA. Besides, the absorption spectra of DNA start decreasing, while titrating by dopamine. The binding constant of dopamine with DNA was calculated and it was shown that for the strong binding this parameter is 1.2×10^5 M^{-1} and for weak binding – 2.3×10^3 M^{-1}. From the data, obtained in this work, one can conclude that DNA may be used as a possible sensitive biomolecule in the dopamine-sensors.

Key words. DNA, dopamine, interaction, UV-melting, absorption.

Introduction.

Dopamine is a small catecholamine molecule, which has an irreplaceable importance in the central nervous system. It is a neuroactive amine in the catecholamine synthesis chain (noradrenaline, adrenaline). Via special enzymes, dopamine is synthetized from L-tyrosine and immediately from Levodopa in the middle brain neurons and adrenal glands, then consequently through -hydroxylase dopamine turns to noradrenaline in the adrenal glands, after which the last one turns into adrenaline through N-methyl-transferase. Being synthetized in the middle brain, dopamine, via dopaminergic pathways, gets to the subcortical and cortical regions of the big hemispheres and regulates number of important functions. From the middle brain, dopamine gets to the frontal cortex, limbic system and subcortical nuclei through three dopaminergic pathways. In the frontal cortex, the functioning of dopamine is connected to the number of the supreme psychological actions, such as recognition, memory, attention, emotional behavior, learning processes. The dopamine circuit block in this pathway contributes to schizophrenia, bipolar depression, major depressive disorder, etc. [1-4]. However, it is shown that in the patients with schizophrenia, the dopamine level in these regions is sufficiently high. On the other hand, dopamine affects human working memory in the prefrontal cortex. Though, this influence of dopamine is not properly paid to attention. There are few studies, devoted to this problem [5,6], as well as experiments on the nature of recognition of the emotional valence of visual stimuli by carriers of different Val/Val and Val/Met genotypes [7]. Nowadays, the role of prefrontal dopamine in working memory and cognitive control is intensively researched [5,8-10].

In the limbic structures, dopamine controls pleasure, orientation, addiction, emotions and perception. Dopamine pathway block in this region results in sharp decreasing of strengthening role of emotions in the learning processes. In the subcortical nuclei, the functioning of dopamine is connected to the movement and sensory. Dopamine block in these pathways results in development of Parkinson's disease. Thus, dopamine level alterations in the brain are immediately bound to the changes in psycho-emotional state, including depression, stress and anxiety. Nowadays, it is evidenced that disorders in the human mental health are connected not only to the various leisure and injuries in the nervous centers, but also to the functional connection deviations. Dopamine, noradrenaline, adrenaline, serotonin, acetylcholine are considered to be the most important neurotransmitters in the brain and can serve as an important biomarker for different pathological situations [1,11].

Thus, the monitoring of the concentration of these neurotransmitters will give an opportunity to make detectable many mental and movement diseases at original states of the new developing disease. In the contemporary diagnostic

methods, it is intensively elaborated new techniques to detect the concentrations of dopamine, serotonin, noradrenaline, adrenaline, acetylcholine, etc. Traditional detecting methods include electrochemical sensors [12], which allow to detect the material concentration in real time, during several milliseconds. There exist many works, devoted to the various detection techniques to monitor dopamine concentration in vivo, among them electrochemical, optical, etc. [1,2,12-21]. However, the application of biosensors can be an appropriate solution for the dopamine concentration determination. The underlayer, which should be used in the biosensor for dopamine binding and its concentration determination, should be a macromolecule, binding to dopamine. Since dopamine in the biological fluids may be bound with the proteins, it is convenient to use nucleic acids as an underlayer. Among nucleic acids, particularly, DNA may be used. There are several works in the literature, insisting that DNA may bind to dopamine [22-24].

Nonetheless, dopamine solution is used in medicine for increasing cardiac pumping strength and blood flow to the kidneys in stressful conditions. On the other hand, dopamine leads to water amount increasing in the human organism. Because of dopamine adverse effects, water was proposed to purify from dopamine contamination [1,25].

In this work, the dopamine interaction with DNA is studied, with the further purpose to apply this interaction as the basis of creation of dopamine sensors with DNA-underlayer.

Materials and Methods.

In the experiments, calf thymus DNA, dopamine hydrochloride ("Sigma-Aldrich", USA), physiological solution were used. Concentrations of DNA and dopamine were determined spectrophotometrically, using the following values of the extinction coefficients: ε_{260}=6600 $M^{-1}cm^{-1}$ for DNA and ε_{280}=2200 $M^{-1}cm^{-1}$ for dopamine hydrochloride. The experiments were carried out at the ionic strengths of the solution 0.02 M and 0.01 M Na^+. The medium pH was equal to 7.0.

UV-melting measurements: The samples of DNA and dopamine solutions were placed in the cells in UV-VIS spectrophotometer Perkin Elmer Lambda 365 (Netherlands) and the temperature of the solutions was being increased in cuvettes by temperature controlling equipment up to 90^0C, with the rate 0.5^0C/min. The concentration ratio dopamine/ DNA of the complexes was equal to 1/5, 1/2 and 1/1. Thermal denaturation started from 50^0C up to 90^0C. At each increment (after each 1 minute), the values of the temperature and respective absorption were fixed on PC. The absorption value was fixed at the wavelength λ=260 nm. Obtaining the total data, the denaturation curves were constructed – dependence of denaturation degree (1-θ) on temperature (t, ^{0}C). The methodology of denaturation curve construction is described in [26]. From the denaturation curves the denaturation parameters were determined – denaturation degree (T_m) and denaturation interval width (ΔT). From the change of these values, one can judge about the thermostability alteration of the complexes.

Absorption spectroscopy measurements: The absorption measurements were carried out on the spectrophotometer Perkin Elmer Lambda 365 (Shelton, CT, USA), using quartz cuvettes with hermetically closing Teflon caps with optic pathway length 1 cm and volume 3 ml. The absorption spectra of dopamine and the complex dopamine-DNA were obtained in the interval 220-350 nm, while the solution of dopamine was titrated by the solution of DNA, the concentration ratio of dopamine/ DNA changed from 1/5 to 1/1. From the absorption spectra, the binding curve was constructed and the binding constant (K) was calculated. It was calculated, according to Scatchard's equation:

$$(1/C_{free})\times(C_{bound}/C_{DNA}) = nK - K(C_{bound}/C_{DNA}) \quad (1)$$

where C_{bound} and C_{free} are concentrations of bound and free dopamine respectively, C_{DNA} is DNA total concentration, K – binding constant and n – number of binding sites. The dopamine free and bound concentrations were calculated, using the maximal absorption value at the fixed wavelength.

The statistical analysis was carried out by the method of Student.

Results.

The melting curves of DNA and DNA-dopamine complexes are presented in the figure 1. In the figure 1a, the melting curves of DNA and the complexes dopamine-DNA are presented at the ionic strength of the solution 0.02 M Na+ and in the figure 1b – that at the ionic strength of the solution 0.01 M Na^+.

As it is obvious from figure 1a, dopamine stabilizes the structure of DNA, which is reflected by the melting curve shift toward high temperatures. From the melting curves, the melting temperature was determined. The values of the melting temperatures are presented in the Table 1.

r=0	**r=1/5**	**r=1/2**	**r=1/1**
Ionic strength of the solution 0.02 M Na^+			
T_m	69	72.9	73.8	73.8
Ionic strength of the solution 0.01 M Na^+			
T_m^1	63	63	65	67
T_m^2	63	75	79	82
T_m^3	63	82	86	88

From the table data, it is obvious that dopamine binding to DNA results in increasing of the melting temperature of DNA. It indicates that there exists an interaction between DNA and dopamine. On the other hand, the melting interval width of DNA is equal to 16°C and 10°C at the ionic strengths of the solution 0.02 and 0.01 M Na^+ respectively, while in the case of the complexes dopamine-DNA it is widening, which also indicates the stabilization of DNA by dopamine. The melting interval widths of DNA-dopamine complexes are not calculated, since these melting curves are not monophasic, which indicates that dopamine binds with DNA in more, than one region. Even from the shape of the melting curves of the complexes, it is obvious that they are biphasic and triphasic, indicating that there exist, at least, two and three binding regions on DNA for dopamine.

To confirm the results, obtained by UV-melting method, the absorption spectroscopy method was applied. In the figure 2, the absorption spectra of DNA and its complexes with dopamine are presented at the ionic strength of the solution 0.02 M Na^+. As it is obvious from the figure 2, the absorption maxima of DNA at λ=260 nm are decreasing along with titration with dopamine solution. On the other hand, dopamine has an absorption maximum at λ=280 nm, however, because

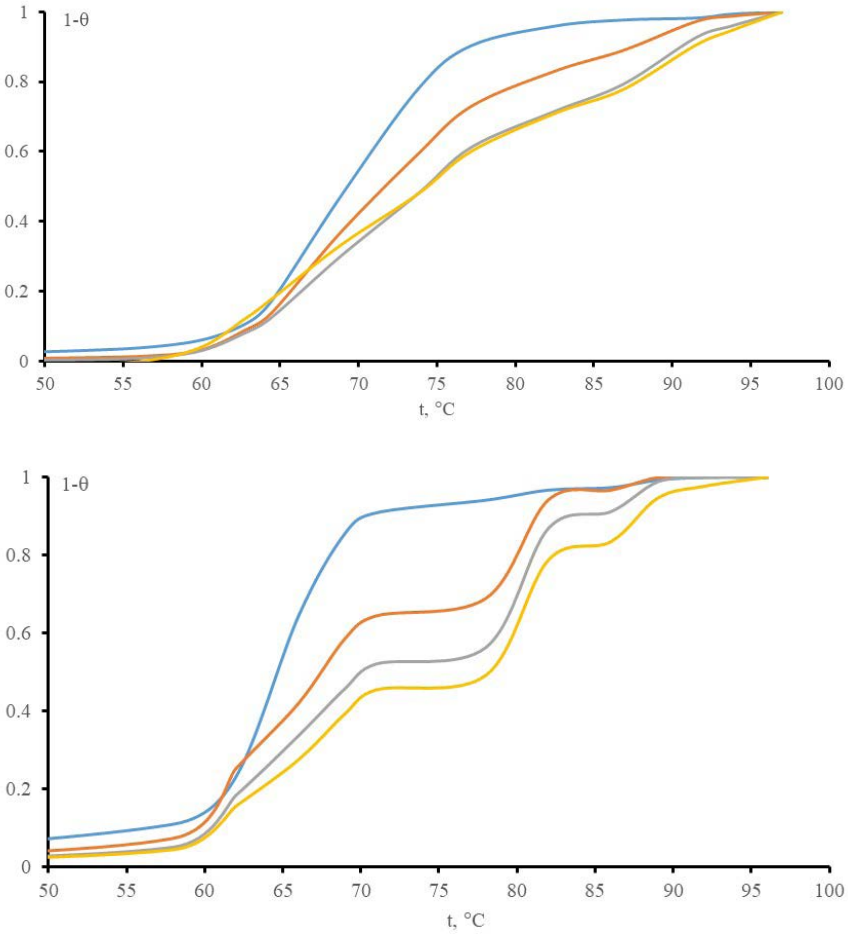

Figure 1. *a – Melting curves of DNA and DNA-dopamine complexes. The curve 1 corresponds to pure DNA melting curve; the curve 2 corresponds to the melting curve of DNA-dopamine complex at the dopamine/DNA ratio 1/5; the curve 3 – 1/2; and the curve 4 – 1/1. The ionic strength of the solution was equal to 0.02 M Na^+.*
b – Melting curves of DNA (1) and DNA-dopamine complexes. The curve 1 corresponds to pure DNA melting curve, the curve 2 corresponds to the melting curve of DNA-dopamine complex at the dopamine/DNA ratio 1/5, the curve 3 – 1/2; and the curve 4 – 1/1. The ionic strength of the solution was equal to 0.01 M Na^+.

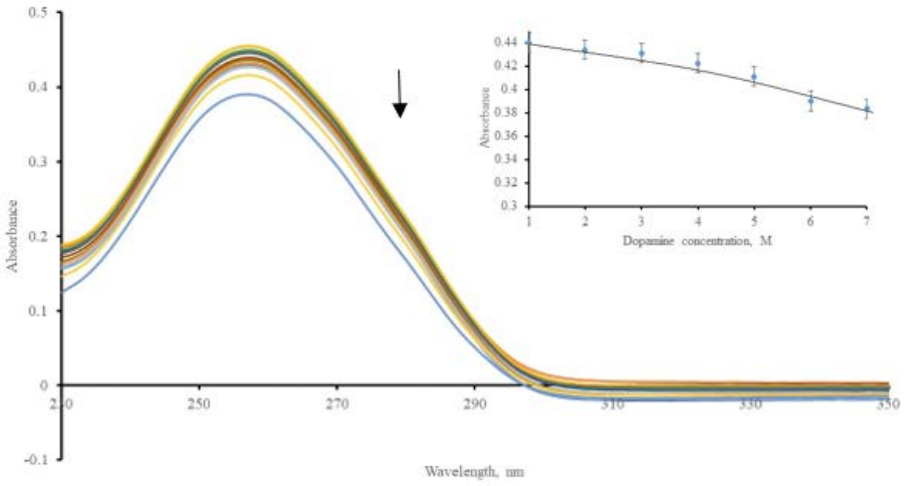

Figure 2. *Absorption spectra of DNA and the complexes dopamine-DNA at the ionic strength of the solution 0.02 M Na^+.*

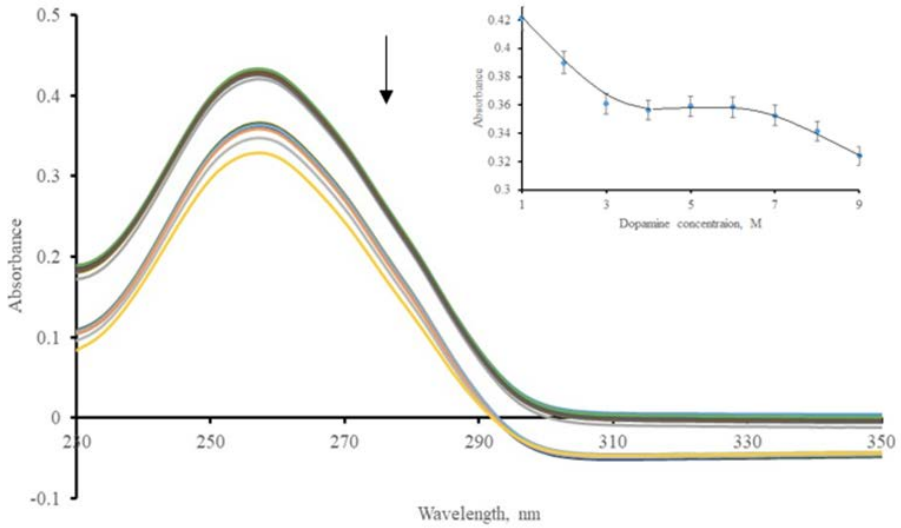

Figure 3. *Absorption spectra of DNA and the complexes dopamine-DNA at the ionic strength of the solution 0.01 M Na^+.*

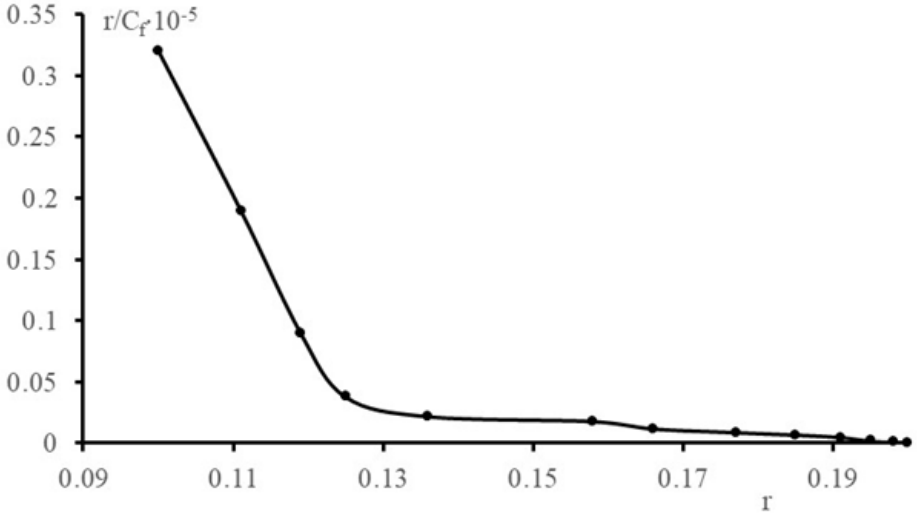

Figure 4. *The binding curve of dopamine to DNA in Scatchard's coordinates at the ionic strength of the solution 0.01 M.*

the dopamine concentrations in the presence and the absence of DNA are similar, at the wavelength 280 nm, the peaks are not formed. Since the absorption of bound dopamine is smaller, than that for free one, the absorption maxima go to the negative absorptions out of DNA and dopamine absorption layer (λ≥300 nm). Moreover, dopamine binding to DNA leads to decreasing of the transmitter free molecules, due to which a decrease of the absorption at the wavelength 260 nm takes place. In the input figure, the dependence of DNA absorption maxima on the dopamine concentration is performed.

It was shown that at the ionic strength of the solution 0.02 M, the decrease of absorption maximum is pronounced. It is obvious from the input figure in the figure 2 that the absorption maximum of DNA decreases linearly along with addition of dopamine. In the figure 3, the absorption spectra of dopamine and its complexes with DNA are presented at the ionic strength of the solution 0.01 M. Here also a decrease of DNA absorption maxima occurs along with titration by dopamine solution. In the input figure 3, the dependence of DNA absorption maxima on the dopamine concentration is performed. It was shown that at the ionic strength of the solution 0.01 M, the decrease of absorption maximum is not linear, but with two grades. This fact maintains that, at low ionic strengths of the solution, dopamine binding to DNA is more pronounced. In the figure 4, the binding curve was constructed, in Scatchard's coordinates.

From the binding curve (Figure 4), the binding constants were calculated. It was shown that for the strong mode of the binding $K=1.2\times10^5$ M^{-1} and for the weak – electrostatic interaction $K=2.3\times10^3$ M^{-1}. From the value of the binding constant for strong interaction, and from melting data, as well as based on the literature data, one can note that the interaction, most apparently, takes place by the mode of intercalation [24,27].

Discussion.

In this work, the binding of dopamine with DNA has been explored to find out the opportunity of DNA application as an underlayer in dopamine-biosensors, aimed at revealing dopamine concentration alterations in the blood and urine. The interaction was studied by the methods of UV-melting and absorption spectroscopy. From the melting curves, presented in the figure 1, one can notice that in comparison to the DNA melting curve, those for the complexes dopamine-DNA are shifted toward higher temperature region. Thus, the melting temperature of the complexes DNA-dopamine increases, compared with the DNA melting temperature. Moreover, the value of the melting temperature for the complexes dopamine-DNA also rises with the enhancement of the ratio dopamine/DNA. On the other hand, the melting curve shape, i.e. interaction type depends on the ionic strength of the solution. Thus, it is obvious from figure 1a that along with concentration ratio enhancement dopamine/DNA, at the ionic strength of the solution 0.02 M Na^+, the shapes of the melting curves turn to be biphasic. It indicates that at the mentioned ionic strength of the solution there exist, at least, two binding regions on DNA for dopamine. From the figure 1b, it is obvious that at the ionic strength of the solution 0.01 M Na^+, along with dopamine/DNA concentration ratio enhancement, the shapes of the melting curves become triphasic, indicating that there are, at least, three regions of the interaction. Whether, at the ionic strength 0.02 M Na^+, DNA melting temperature is equal to 69°, it increases by 3.9°C for the dopamine/DNA ratio r=1/5 and by 4.8°C for the ratios r=1/2 and 1/1. These experimental results indicate that there exist a significant stabilization of DNA, induced by dopamine binding. On the other hand, the melting curve shapes for the complexes dopamine-DNA are biphasic, while the melting curve shape for pure DNA is monophasic. This experimental fact indicates that dopamine interacts with DNA in more, than one region. Consequently, one can presume that there may be two interaction modes – strong and weak.

Similarly, discussing the figure 1b, one can notice that whether the melting temperature of pure DNA, at the ionic strength of the solution 0.01 M Na^+, is equal to 63°C, it increases by 6°C for the dopamine/DNA ratio r=1/5, by 8°C for the ratio r=1/2 and by 11°C for the ratio r=1/1. These results indicate that the dopamine stabilizes DNA structure at the high concentration ratios, however, the stabilization is stronger at 0.01 M, than for the case of that at the ionic strength of the solution 0.02 M Na^+. The second peculiarity is that the shapes of the melting curves of the complexes dopamine-DNA are triphasic, indicating that at the ionic strength of the solution 0.01 M Na^+, there exist, at least, three regions of binding. From the figure 1b, it is also obvious that the melting curves of the complexes dopamine-DNA are triphasic that is why we cannot determine one value for the melting temperature. Proceeding from this fact, we have divided the melting curve into three regions. We assume that the below part of the melting curve corresponds to the dopamine binding to AT-rich sequences, forming hydrogen bonds. The middle part is the main binding region, where the strong binding mode is thought to exist. The top part of the melting curve corresponds to the GC-rich region binding. Considering these assumptions, for the melting curves of the complexes dopamine-DNA at the ionic strength 0.01 M, three values for the temperatures were determined and presented in the Table 1. It is obvious from the table data that the melting temperature of DNA is decreasing with the decrease of the ionic strength of the solution. Besides, the melting temperature increases along with dopamine/DNA concentration ratio enhancement for three regions in the melting curves of the complexes dopamine-DNA at the ionic strength 0.01 M.

Summarizing the experimental data, obtained by the method of UV-melting, we assume that depending on the ionic strength of the solution, dopamine, at high concentration ratios, binds to DNA in more, than one region and consequently, by more, than one mode. Remarkably, there exist two and three interaction regions in the case of high ratios of dopamine/DNA, because at the ratios, lower, than 1/5 occurs weak binding, possibly by only one binding mode. At the ionic strength of the solution 0.02 M Na^+, dopamine interacts with DNA, by strong and weak – possibly electrostatic modes [25]. Though, it is not excluded that with these regions dopamine binds by more, than one mode, among them the one mode is electrostatic interaction. Taking this fact into account, biphasic or triphasic melting curves are not possible to explain from the point of existence of only weak interaction mode. Based on this, we assume that apart from the weak – electrostatic interaction, dopamine interacts with DNA by the other modes as well. In all appearances, the strong binding mechanism of dopamine to DNA is intercalation [24], though; the outside amino-groups form hydrogen bonds with DNA nucleotides. This fact leads to the melting temperature enhancement of the complexes, as well as induces a heterogeneous melting of the different regions of DNA. We assume the following process of dopamine binding to DNA at this ionic strength of the solution: at first, dopamine binds to DNA AT-rich regions by hydrogen bonds, then, it stabilizes by the strong binding mode. Later, when these regions start melting, dopamine binds to other regions, including GC-rich satellites and stabilizes them. It is not excluded that during the first phase, the ring of dopamine is localized in the minor groove; amino groups form hydrogen bonds with adenine [25]. During the second phase, it binds to the random nucleotide sequence in the same mode.

To maintain this presumption, the study was carried out by the method of absorption spectroscopy. In the figure 2 and 3, the absorption spectra of DNA were obtained without dopamine, then along with titration by dopamine. As it is obvious from figures 2 and 3, the spectra decrease along with addition of dopamine to DNA solution. The total decrease in absorption value at the wavelength 260 nm is pronounced at the ionic strength of the solution 0.02 M Na^+, and more pronounced at the ionic strength of the solution 0.01 M. These data obtained indicate that at relatively higher ionic strength of the solution the absorption decreases less, than at the lower ionic strength of the solution. Most apparently, it is connected to the fact that at low ionic strengths dopamine binds stronger to DNA, than at high ionic strength of the solution. This fact also indicates that there are two or more types of interaction between dopamine and DNA at high concentrations ratio. It is remarkable that the

results, obtained by the absorption measurements, confirm those, obtained by UV-melting method. From the absorption spectra, the values of the binding constants were calculated. The value of K for the strong mode of the binding – 1.2×10^5 M^{-1} indicates the strong binding mode of the interaction of dopamine with DNA, while the value of K for the weak interaction – 2.310^3 M^{-1} indicates that there is also a weak – electrostatic binding, which is natural for mostly protonated dopamine and polyanion DNA.

Conclusion.

Thus, the results, obtained in this work, indicate that dopamine interacts with DNA at least in more, than one regions and possibly by more, than one mode – strong and weak – electrostatic interaction. Though, at low ionic strength of the solution, the third binding region on DNA for the dopamine is appeared. The data, received by the UV-melting method, show that DNA melting curves are shifted toward higher temperature region, along with dopamine interaction with DNA. Although dopamine leads to DNA stabilization, this effect is detectable at the high ratios of dopamine/DNA – equal to 1/5, 1/2 and 1/1. DNA melting temperature increases along with dopamine binding to it, as compared to that for pure DNA. On the other hand, the melting curves of the complexes dopamine-DNA acquire biphasic shape at the ionic strength of the solution 0.02 M Na^+, and triphasic shape – at the ionic strength of the solution 0.01 M Na^+, while the melting curve of DNA is monophasic. This fact experimentally insists on the existence of more, than one binding mode of dopamine to DNA. To confirm these assumptions, the absorption spectra of DNA were registered, while titrating by dopamine. The absorption spectra of DNA decrease along with titration by dopamine. Thus, we assume that at low ionic strengths of the solution, dopamine is less screened by anti-ions from DNA, compared to high ionic strengths of the solution. That is why at low ionic strengths of the solution dopamine can easily enter to GC-rich regions in DNA, since these regions come out to major, more hydrophilic groove, as well as DNA owns more relaxed (less compact) conformation at low ionic strengths of the solution.

Thus, the experimental results, obtained in this work, ensure that DNA may be used as a sensitive biomolecule for the dopamine-detection in biological fluids, since dopamine possesses a sufficiently high affinity toward nucleic acids. It is remarkable, that the affinity of dopamine toward DNA depends on the ionic strength of the solution. Taking into account that dopamine concentration is higher in the urine, than in the blood [1,11], we can propose to apply the urine as a biological fluid to make an express test. Based on the above mentioned, we assume that among biological fluids in the human organism, urine may be used as the most appropriate material for revelation of dopamine concentration, with the application of nucleic acids.

Author Contributions.

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Conflict of Interest.

The authors declare that there is no any conflict of interest.

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