МІНІСТЕРСТВО ОСВІТИ І НАУКИ УКРАЇНИ
НАЦІОНАЛЬНИЙ АВІАЦІЙНИЙ УНІВЕРСИТЕТ
ФАКУЛЬТЕТ ЕКОЛОГІЧНОЇ БЕЗПЕКИ, ІНЖЕНЕРІЇ ТА ТЕХНОЛОГІЙ
КАФЕДРА БІОТЕХНОЛОГІЇ

ДОПУСТИТИ ДО ЗАХИСТУ
Завідувач випускної кафедри
_____ М.М. Барановський
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ДИПЛОМНА РОБОТА

(ПОЯСНЮВАЛЬНА ЗАПИСКА)

ЗДОБУВАЧА ВИЩОЇ ОСВІТИ ОСВІТНЬОГО СТУПЕНЯ «БАКАЛАВР»
СПЕЦІАЛЬНІСТЬ 162 «БІОТЕХНОЛОГІЇ ТА БІОІНЖЕНЕРІЯ»
ОСВІТНЬО-ПРОФЕСІЙНА ПРОГРАМА «ФАРМАЦЕВТИЧНА БІОТЕХНОЛОГІЯ»

Тема: «Розробка хелатокомплексів на основі фулерену, що зменшують стресові стани м'язової системи»

Виконавець: студентка ФБ 404 групи Гончаренко І.І.

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Нормоконтролер: Дражнікова А.В.

КИЇВ 2021

MINISTRY OF EDUCATION AND SCIENCE OF UKRAINE
NATIONAL AVIATION UNIVERSITY
FACULTY ENVIRONMENTAL SAFETY, ENGINEERING AND TECHNOLOGY
DEPARTMENT OF BIOTECHNOLOGY

ALLOWED TO DEFENCE
Head of the graduate department
_____M.M. Baranovsky
«___»_____2021

BACHELOR THESIS

(EXPLANATORY NOTE)

OF APPLICANT OF EDUCATIONAL DEGREE «BACHELOR»
SPECIALTY 162 «BIOTECHNOLOGY AND BIOENGINEERING»
EDUCATIONAL PROFESSIONAL PROGRAM «PHARMACEUTICAL BIOTECHNOLOGY»

Theme: «Development of fullerene-based helatocomplexes , that reduce stress in the muscular system»

Executor: student of group 404a, FESET — Honcharenko I.I.

Supervisor of studies: PhD in chem. Assoc. Prof. — Mykhailenko O.V.

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НАЦІОНАЛЬНИЙ АВІАЦІЙНИЙ УНІВЕРСИТЕТ

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Спеціальність 162 «Біотехнології та біоінженерія»

Освітньо - професійна програма «Фармацевтична біотехнологія»

ЗАТВЕРДЖУЮ

Завідувач випускної кафедри

_____ М.М. Барановський

«___»_____2021 р.

ЗАВДАННЯ

на виконання дипломної роботи

Гончаренко Ірини Ігорівни

1. Тема дипломної роботи: «Розробка хелатокомплексів на основі фулерену, що зменшують стресові стани м'язової системи» затверджена наказом ректора від «11» травня 2021 р. № 715/ст.

2. Термін виконання роботи: з 10 травня по 14 червня 2021 р.

3. Зміст пояснювальної записки: ВСТУП; ЛІТЕРАТУРНИЙ ОГЛЯД; МАТЕРІАЛИ ТА МЕТОДИ ДОСЛІДЖЕНЬ; РОЗРОБКА ХЕЛАТОКОМПЛЕКСІВ НА ОСНОВІ ФУЛЕРЕНУ, ЩО ЗМЕНШУЮТЬ СТРЕСОВІ СТАНИ М'ЯЗОВОЇ СИСТЕМИ; ВИСНОВКИ; СПИСОК БІБЛІОГРАФІЧНИХ ПОСИЛАНЬ ВИКОРИСТАНИХ ДЖЕРЕЛ.

4. Перелік обов'язкового графічного (ілюстративного матеріалу): 2 таблиці ,14 рисунків.

5. Календарний план-графік

№	Завдання	Термін виконання	Відмітка про виконання
1	Вибір теми дипломної роботи	11.05.2021	
2	Огляд та збір інформації за темою дипломної роботи: «Розробка хелатокомплексів на основі фулерену, що зменшують стресові стани м'язової системи»	12.05.2021	
3	Написання першого розділу дипломної роботи	14.05.2021	
4	Написання другого розділу дипломної роботи	17.05.2021	
5	Написання третього розділу дипломної роботи	22.05.2021	
6	Написання висновків	26.05.2021	
7	Підготовка презентації	28.05.2021	
8	Огляд дипломної роботи керівником	29.05.2021	
9	Попередній захист дипломної роботи	02.06.2021	
10	Захист дипломної роботи	14.06.2021	

6. Дата видачі завдання: «__11___»__травня___2021 року

Керівник дипломної роботи _____ Михайленко О.В.

(підпис керівника)

Завдання прийняв до виконання _____ Гончаренко І.І.

(підпис випускника)

NATIONAL AVIATION UNIVERSITY

Faculty Environmental Safety, Engineering and Technologies

Department of Biotechnology

Speciality: 162 «Biotechnology and bioengineering»

EPP: «Pharmaceutical Biotechnology»

APPROVED

Head of the Department

_____Baranovsky M.M.

«___» _____2021

TASK

From bachelor thesis of student

Honcharenko Iryna Igorivna

1. Theme of degree work: « Development of fullerene-based helatocomplexes that reduce stress in the muscular system » approved by the Rector «11» of May 2021 № 715/ст
2. Term of work execution: from 10 of May 2021 and 14 of June 2021.
3. Contents of the explanatory note: INTRODUCTION; LITERARY REVIEW; MATERIALS AND RESEARCH METHODS; DEVELOPMENT OF FULLERENE-BASED HELATOCOMPLEXES THAT REDUCE STRESS STATES OF THE MUSCLE SYSTEM; CONCLUSIONS; LIST OF BIBLIOGRAPHICAL REFERENCES OF SOURCES USED.
4. List of obligatory graphic (illustrative material): 2 tables, 14 figures.
5. Schedule.

№	Task	Execution term	Signature of the head
1	Choice of thesis topic	11.05.2021	
2	Literature review and information collection on the topic of thesis: " Development of fullerene-based helatocomplexes that reduce stress in the muscular system "	12.05.2021	
3	Writing the first section of the thesis	14.05.2021	
4	Writing the second section of the thesis	17.05.2021	
5	Writing the third section of the thesis	22.05.2021	
6	Writing conclusions	26.05.2021	
7	Preparing a presentation	28.05.2021	
8	Review of the thesis by the thesis supervisor	29.05.2021	
9	Preliminary defense of the thesis	02.06.2021	
10	Defence of graduating work.	14.06.2021	

6. Date of task receiving: «11» of May 2020

Supervisor of degree work _____ Mykhailenko O.V.

(signature of the head)

Task for execution was taken over by_____Honcharenko I.I.

(graduate's signature)

РЕФЕРАТ

Пояснювальна записка до дипломної роботи «Розробка хелатокомп
основі фулерену ,що зменшують стресові стани м'язової системи.»: 49 сторінки, 10 рисунків, 55 використаних літературних джерел.

Мета роботи: вивчити структуру комплексів фулерена С60 з лактатами деяких металів при нагріванні методами молекулярної динаміки , розрахувати УФ-спектри комплексів залежно від концентрації лактатів та визначити константи асоціації систем.

Завдання роботи:

1.Здійснити огляд літератури.

2 Пошук ефективних препаратів (дизайн), включаючи комплекси біосумісних наноструктур з можливістю таргентної доставки, які б дозволилимаксимально зменшувати патологічні наслідки травматичних ушкоджень скелетних м’язів. 3.Теоретично промодельовати лактати металів, фулерени у водній суспензії, комплекси лактатів металів з фулереном С60 та їх вплив на м'язову активність. 4.Оцінити перспективність терапевтичного впливу створеного препарату на швидкість післястресового відновлення нативних механокінетичних характерсистик м’язової системи.

Обєкт дослідження: Розробка хелатокомплексів на основі фулерену , що зменшують стресові стани м'язової системи.

Предмет дослідження: комплекси лактатів металів з фулереном С60.

Методи долідження: аналітичні, теоретичні , статистичні.

Результати дипломної роботи можуть бути використані під час досліджень та на практиці спеціалістів-біологів та спеціалістів-біотехнологів.

ГЕЛАТОКОМПЛЕКСИ НА ОСНОВІ ФУЛЛЕРЕНІВ, М'ЯЗОВА СИСТЕМА, ЛАКТАТИ, С60 ФУЛЛЕРЕНИ, ФУЛЛЕРЕННО-ЛАКТАТНА СИСТЕМА, КВАНТОВО-ХІМІЧНИЙ ДИЗАЙН.

ABSTRACT

Explanatory note to the thesis "Development of helatocomplexes based on fullerene, reducing stress in the muscular system.": 49 Pages,10 figures, 55 references.

Purpose: to study the structure of C60 fullerene complexes with lactates of some metals when heated by molecular dynamics, to calculate the UV spectra of complexes depending on the concentration of lactates and to determine the association constants of the systems.

Tasks of work:

1. Review the literature**.**

2 Search for effective drugs (design), including complexes of biocompatible nanostructures with the possibility of targeted delivery, which would minimize the pathological consequences of traumatic skeletal muscle injuries.

3. Theoretically model metal lactates, fullerenes in aqueous suspension, metal lactate complexes with C60 fullerene and their effect on muscle activity.

4. To evaluate the prospects of therapeutic effect of the created drug on the rate of post-stress recovery of native mechanokinetic characteristics of the muscular system.

Object of research: Development of helatocomplexes on the basis of fullerene that reduce stress states of the muscular system.

Subject of research: complexes of metal lactates with C60 fullerene.

Research methods: analytical, theoretical, statistical.

The results of the thesis can be used during research and in the practice of specsalists-biologast and specialists-biotechnologists.

FULLERENE-BASED HELATOCOMPLEXES, MUSCULAR SYSTEM, LACTATES, C60 FULLERENES, FULLERENE-LACTATE SYSTEM, QUANTUM-CHEMICAL DESIGN.

CONTENT

INTRODUCTION

According to the WHO, there is an annual increase in injuries in the world, the main causes of which are road accidents (accidents), domestic and military-caused injuries. Skeletal muscle injuries, accompanied by tissue ischemia in many cases, are the cause of severe pathologies with high mortality and one of the main causes of post-traumatic pathologies after surgery. The main goal in the treatment of these pathologies is the rapid recovery of physiological metabolism in the damaged areas. However, current therapeutic techniques are not very effective due to the low bioavailability of metabolic modulators for damaged tissues. A promising solution to this problem is the use of biotechnological approaches aimed at modulating the activity of natural components of alkaline earth metal lactates, which in bioavailable nanocomplexes are able to purposefully increase the stress resistance of the muscular system. This will not only improve existing therapeutic strategies for the correction of traumatic conditions, but also significantly accelerate post-traumatic recovery. Thus, the aim of the thesis is to develop technologies for creating a bioavailable form of nanocomplexes based on fullerenes with metal lactates and their use in vivo to increase the effectiveness of trauma therapy of the muscular system.

A fundamentally important element is the use of the original method of stimulation of individual muscles (as close as possible to physiological processes) and the simultaneous fixation of several mechanokinetic parameters of muscle activity for a detailed analysis of its dynamics. It is planned to use a technique of artificial initiation of pathological changes, similar to the immobilization of skeletal muscles, similar to that which accompanies the treatment of traumatic injuries. In addition, different durations of pathological factors were modeled and the therapeutic effect of the studied compounds was studied. The new results were obtained in experiments with simultaneous registration of the activity of motor units in the antagonist muscles of the injured joint during isotonic movements and the generation of isometric forces. Different types of muscle pathologies have been studied for sensitivity to various therapeutic methods of using lactate metal complexes with a system of targeted transport - fullerenes (different time of administration

relative to the time of initiation of the pathological process, different methods of in vivo administration and dosing). Studies of the rate of recovery of motor functions after cessation of pathological factors reveal modifying effects of the studied drugs not only on the pathogenesis of post-traumatic conditions, but also on the rate of recovery of motor function, which is a necessary prerequisite for recovery. Since basic research on the effect of lactates of metals with a system of targeted transport on the pathogenesis of conditions induced by traumatic skeletal muscle injury is currently lacking, the proposed work is new and relevant in the field of further biomedical applications.

The relevance of the choice of therapeutic drugs, namely the choice of coordinating metal, lipo- and hydrophilicity of ligands (actually substituents - amino, hydroxy, alkyl, aryl, getaryl, etc.) to study the formation of complexes with lactate and immobilization transport component - fullerenes, is due a number of factors. For example, magnesium is a cofactor of many enzymatic reactions. Magnesium is needed to convert creatine phosphate to ATP, a nucleotide that is a universal source of energy for muscle cells. Magnesium is required at all stages of protein synthesis, has a vasodilating effect and reduces blood viscosity. All these properties make it an extremely important agent for the treatment of traumatic injuries of the muscular system and their local and generalized consequences, and an even more important agent for the post-traumatic restoration of physiological muscle condition. Another trace element - zinc is characterized by an inverse relationship of its content in the blood with acidification of the body during muscle work and a direct relationship with testosterone levels in experiments on rats. Zinc increases exercise tolerance and accelerates myocyte repair after skeletal muscle damage. In general, all the micronutrients selected for the study have common features:

- impossibility of self-assimilation in the intestine;
- the ability to form stable and well absorbed by the body complexes;
- increase the stress resistance of the muscular system Therefore, the aim of the thesis is to design targeted drugs to correct pathological conditions of traumatic origin, accelerate the recovery of the muscular system after motor dysfunction caused by these conditions, and reduce long-term pathological motor disorders.

CHAPTER 1
LITERATURE REVIEW

1.1. Functioning of the muscular system and development of pathological processes

The establishment of molecular mechanisms of skeletal muscle contraction in their pathological damage largely depends on an adequate assessment of changes in their mechanokinetic characteristics. The fundamental difference between the mechanokinetic properties of muscle contraction and relaxation, as well as the significant dependence of dynamic characteristics on the direction of movement significantly complicates the experimental analysis of these processes. The relationship between frequency coding and actual patterns of efferent activity of even intact muscle units has not yet been studied in detail. The study of the therapeutic effect of nanocomplexes against the background of general dysfunction of the muscular system due to the development of the pathological process is also a difficult problem, because it is known that the effect of drugs on the muscular system is not constant, but depends on muscle contraction. strength, degree of fatigue, the possibility of targeted delivery of the active substance, its half-life and possible toxic side effects.

Among the pathologies that develop in skeletal muscles during injuries, ischemic injuries account for more than 35% of the total number of injuries of the musculoskeletal system. The consequences of ischemia depend on the degree and rate of reduction of blood flow parameters, its duration, tissue sensitivity to hypoxia, the general condition of the body. The long-term consequences of traumatic muscle injuries are ischemic contractures resulting from circulatory disorders in muscles, nerves and other tissues, followed by scarring. At present, the issues of ischemia of traumatic origin and the symptoms that accompany it are insufficiently studied. Clinical manifestations of ischemia are unsystematized and quite complex. Only recently have the severity and stages of hypertensive ischemic syndrome and subsequent ischemic contracture been described. A

small number of physiological manifestations that accompany ischemia have been studied and described. The question of the functioning of ischemic limbs remains open. However, it is already well known that the rapid detection of pathology and the beginning of targeted therapy of traumatically induced physiological stress reactions is crucial for the effectiveness of therapy in general, and the subsequent possible level of recovery of motor functions. Therefore, the search for effective drugs (design), including complexes of biocompatible nanostructures with the possibility of targeted delivery, which would minimize the pathological consequences of traumatic skeletal muscle injuries, is an urgent need for modern biomedicine in general and in Ukraine in particular.

Today there is a problem of low efficiency of treatment of physical injuries, because due to afferent signals coming from the affected area, there is a sharp excitation of the nervous system, intensified metabolism, increased adrenaline, rapid breathing, vasospasm, increased endocrine glands - pituitary gland, adrenal glands. The protective properties of the body are quickly depleted, compensatory capabilities fade and the second phase develops - torpid. In this phase there is a suppression of the nervous system, heart, lungs, liver and kidneys. Toxic substances that accumulate in the blood cause paralysis of blood vessels and capillaries. Blood pressure is reduced, blood flow to the organs is sharply reduced, oxygen starvation is intensified - all this can very quickly lead to the death of nerve cells and even death of the victim. Injury treatment is usually a very complex process, from first aid to complex surgery using modern anesthesia instruments. Restoration of the chemical and energy balance of the victim is the main task of therapy. Lactic acid plays an important role in the cycle of energy conversion in the body. However, the authors of [1] identified additional functions of lactate, namely the signaling functions of intercellular interaction. The existence of hitherto unknown lactic acid receptors in brain cells, which are present on norepinephrine-releasing cells, is assumed to provide their sensitivity to lactate. It is noted that the next important task is to identify receptors that mediate this effect, as this may be the basis for the development of new drugs that can block or stimulate the norepinephrine response. Pharmacological regulation of the intensity of norepinephrine release - a fundamental factor in the implementation of brain functions - will open the possibility of practical application of this effect in the

treatment of a number of diseases and conditions such as various types of stress, hypertension, pain and depressive disorders. Currently, lactates are very promising therapeutic substances, in particular coordination compounds based on lactate ligands have perfect transport properties. They can be modified with lipophilic fragments, additionally functionalized with antibodies, polymeric nanocarriers, etc. [2]. According to the authors [3-7], low-toxic and biocompatible carbon nanomaterials and biometals can be used as effective agents in the treatment of pathologies of different genesis. It is proposed to develop complex nanoscale trauma therapy drugs, in particular using fullerenes [8] and lactates of metals, to increase targeted delivery to target cells, prolong their action in the body, increase bioavailability and reduce side effects. It is assumed that the increased energy activity of such drugs is due to both direct effects on injured tissues and indirect effects, in particular the regulation of oxidative stress in the microenvironment of traumatic injury, activation of cellular immunity, etc. [9,10]. The introduction into practice of the created and characterized complex nanopreparations requires experiments both at the cellular level and on animal models with a clear selection and fixation of the dynamics of marker parameters of therapeutic effects.

An important prerequisite for this work is the design of the mechanokinetic activity of both the native muscle and individual motor units and single myocytes under physiological stimulation. In previous years, original methods have been developed to analyze the mechanokinetic characteristics of skeletal muscle contraction with experiments on specially designed electromechanical devices using appropriate servo-controlled hardware and software systems. Effective methods of studying changes in the functioning of the muscular system under the influence of external factors of anthropogenic origin have been established. The proposed experimental approach is based on repeated repetition of the same stereotyped contractions, which can occur both under conditions of appropriate external muscle load and with isometric muscle contraction. The average mechanokinetic parameters of muscle work (strength, length, stiffness, integrated power, duration of tetanic phases, rate of change of force, fatigue) will be used by us to assess the therapeutic and corrective effects of nanocomplexes of fullerenes with metal lactates on normal and pathological muscle conditions. systems. The prospects of the

therapeutic effect of the created drug on the rate of post-stress recovery of native mechanokinetic characteristics of the muscular system will be evaluated.

1.2. The role of fullerenes in modern medicine

The last decade has been characterized by significant achievements in the fields of nanotechnology and nanomedicine. Discovered in 1985, the allotropic form of carbon, the C60 fullerene molecule, is nanoscale, and its structural features determine its unique physicochemical properties. Numerous studies have shown that fullerene (C60) and its derivatives are promising in use due to characteristics such as specific protection against adverse effects on DNA, radioprotective effects, antiviral properties, antioxidant and anti-amyloid effects, angiogenesis inhibition, immunostimulatory and antipyretic positive effect on the restoration of axon growth, use for gene delivery. Given the wide range of applications in medicine, C60-fullerene and its derivatives require further comprehensive comprehensive theoretical and experimental research.

Since 1993, numerous studies have shown that fullerene and its derivatives are of paramount importance in several areas of biology and medicine, such as: specific protection against adverse effects on DNA, ultraviolet and radioprotective effects, antiviral properties, antioxidant and anti-amyloid effects, allergic reactions, inhibition of angiogenesis, immunostimulatory and antitumor effects, positive effect on the restoration of axon growth. Although some independent research groups have confirmed the safety of C60, the toxicity of this fullerene is still a matter of discussion [11-12].

Antioxidant action is one of the main and important properties of C60 fullerene. Study of the intensity of lipid peroxidation (LPO) and the number of astrocyte markers (glial fibrillar protein) in the brain tissues of rats under conditions of long-term consumption (12 weeks) of ethyl alcohol, as well as the study of the protective effects of oral hydrated forms of fullerene C6n (C60Hy rats that received hydrated forms of fullerene compared with the control group [13]. Due to the presence of a system of conjugated double bonds on the surface, the C60 molecule captures free radicals and exhibits antioxidant properties. Thus, water with built-in fullerenes neutralizes free

radicals, ie it is an antioxidant, many times more effective than conventional antioxidants. This effect is due to the fact that water is present in almost all tissues of the body provides access of fullerene to the whole body. Due to the significant concentration of carbon, fullerenes can function as stimulating antioxidants that support the activity of brain neurons, help in the treatment of multiple sclerosis [14].

The possibility of application in oncology is due to the property of fullerenes under the condition of photoexcitation to produce reactive oxygen species and use it as a photosensitizer for anticancer photodynamic therapy.

Today, the idea of creating anticancer drugs based on water-soluble endohedral compounds of fullerenes with radioactive isotopes - fullerene molecules, inside which are placed one or more atoms of another element. Modification of doxorubicin with water-soluble C60 fullerene derivatives has been shown to reduce its toxicity by more than half. The use of C60 in a therapeutic dose increases the effectiveness of treatment to 113%, while this figure for doxorubicin is 58%. It should also be noted that after 60 days with C60 therapy, 17% of mice remained alive, and with doxorubicin therapy, the surviving animals were not observed. Decreased toxicity and increased antitumor activity of the anthracycline antibiotic doxorubicin is observed in its modification with water-soluble derivatives of fullerene C60, which is probably due to their antioxidant properties .

The unique mechanical properties of carbon nanomaterials allow to increase the service life of prostheses in traumatology and orthopedics and improve their wear resistance .

The use of fullerenes in various forms and their introduction into new materials remains an urgent problem. One of such options is the polymerization of fullerenes and their introduction into polymeric materials [15-16].

A new direction in biomedicine is the development of methods for targeted drug delivery using various nanostructures as vectors. It is shown that nanosized forms of carbon (fullerenes, nanotubes) have a high permeability to the biomembrane and, very importantly, can overcome blood-brain barrier and be transporters for drugs. In the future, it can be used to expand the therapeutic possibilities for the treatment of cancer and neurodegenerative diseases, as well as neuroinfections [16].

Limitations for this application are the high degree of hydrophobicity of C60 molecules, agglomeration in an aqueous medium, the formation of nanoparticles of different sizes and, as a consequence, the instability of aqueous dispersions of C60 nanoparticles. He faThe fact that the biological activity of C60-fullerene largely depends on the diameter of the formed nanoparticles, their concentration, surface charge, shape, size distribution, explains a certain inconsistency of data on the biocompatibility and toxicity of C60 fullerene [17].

The addition of pyridines and pyrimidines to fullerene C60 increased their selective neurotropic activity, but increased their overall toxicity by 3-5 times. In vitro experiments should be the basis for better prediction of possible toxicity of nanoparticles and reduce the number of animals used. The researches which subject were toxicity and influence on a state of health of the most widespread kinds of technogenic nanoparticles are considered: carbon nanotubes, fullerenes, metals or their oxides, quantum dots. The cyto- and genotoxicity of nanoparticles, their influence on various systems of an organism, the basic directions of preventive measures at manufacture and application are discussed. The authors concluded that the evidence of toxic effects of carbon nanotubes (suppression of viability and cell death) corresponds to the level "to some extent probable" [18].

Regarding the issue of toxicity, a C60 fullerene study was performed on acute carbon tetrachloride intoxication in rats, a classic model for studying indirect liver damage by free radicals. The results show that an aqueous suspension of fullerene C60, prepared without the use of any polar organic solvent, not only does not cause acute or subacute toxicity in rodents, but it also protects their liver depending on the dose, from the harmful effects of free radicals. According to histopathological examinations and biological tests, fullerene C60 can be considered as a powerful protective agent of the liver [19].

For the first time, transmission electron microscopy has shown that hydrated fullerene C60 inhibits beta-amyloid fibrillation, thus exhibiting its anti-amyloid potential .

1.3. The use of C60 fullerenes as a means of prevention and correction of ischemic disorders in skeletal muscle tissue

Ischemic disorders account for more than 35% of pathologies that develop in skeletal muscle with various injuries [20]. Currently, there is a problem of lack of accurate diagnostic tests to quickly establish the level of ischemic injury, which is crucial for the effectiveness of further therapy [21], because it is a pathological process with rapid development over time: yes, after the first hour of ischemia is reduced to 40% of baseline, and after two hours - by 90%, after which the restoration of the contractile response is observed only in the third week after the rapid restoration of blood flow (reperfusion) [22].

Ischemia is caused by a decrease in arterial blood flow and increased consumption of oxygen and metabolic substrates by tissues. The causes of this disorder can be natural, pathogenic factors and adverse conditions, the impact of which leads to a decrease in the lumen of arterial vessels and difficulty in blood flow through them. Ischemia can be local (individual skeletal muscle) and generalized (simultaneously in different muscles); physiological (temporary and reversible, has an adaptive value. A typical example is ischemia that occurs in skeletal muscles during and after physical training.) and pathological (does not meet the metabolic and functional needs of the muscle, threatens its devitalization) [23]. Ischemically damaged muscles have common features, which include: changes in linear and volumetric blood flow velocity in microvessels, decreased pulsation of arterial vessels, pallor, decreased number of functioning blood and lymphatic microvessels, increased permeability of their walls, decreased muscle turgor and partial pressure of oxygen in the blood, tissue, organs (development of hypoxemia and tissue hypoxia), increased acidosis in the tissue, activation of anaerobic and inhibition of aerobic processes, trophic disorders and decreased tissue or organ temperature, tissue sensitivity (paresthesia, pain), decreased functional activity of both individual myocytes and tissue in general. Reduced blood flow through the arteries reduces the delivery of oxygen, nutrients, and regulators to the muscles. Nerve tissue is most sensitive to ischemia (dies after 5-6 min), muscle tissue tolerates prolonged ischemia (more than 6 h), and bone tissue is most resistant to ischemia [24]. The consequences of skeletal muscle ischemia depend on the

degree of damage to the microcirculatory tract, the level of tissue hypoxia, the amount and ratio of metabolic products, Na +, K +, H + the level of mechanical muscle damage, [25].

Skeletal muscle ischemia can be incomplete (developing ischemic stasis, malnutrition and atrophy, dystrophy) and progressive and complete (necrobiosis, necrosis, and scarring occur first). The consequences of disorders depend on the following factors: the degree of development of collateral circulation (well developed in the muscles of the extremities; relative insufficiency is noted in the heart muscle), localization and different sensitivity of tissue or organ to hypoxia (proven particularly high sensitivity to oxygen deprivation). myocardium) duration of ischemia and hypoxia of tissues, diameter of the affected arterial vessel vascularizing the muscle (when closing the lumen of a larger vessel there are significant necrotic tissue lesions).

All morphological factors that contribute to the development of ischemia are divided into deterministic (features of the anatomical structure of the soft skeleton of the forearm - the presence of dense fascial sheaths, the degree of muscle development) and individual (direction of displacement of bone fragments, types of primary and secondary muscle lesions and vascular-nervous bundles).

During skeletal muscle ischemia, there is a high correlation between the duration of ischemia and the subsequent viability of the muscle fiber [26]. Different types of fibers reveal significant metabolic and functional differences. This does not significantly affect their tolerance to injuries from ischemia-reperfusion [27].

Ischemia at the cellular level is characterized by the migration of neutrophils in endomysias and then in perimysias 24 h after 2 h of ischemia and subsequent reperfusion [27]. The immune system plays an important role in restoring muscle function after ischemic injury. There was a correlation between the recovery time of skeletal muscle after ischemic injury with the activity of macrophages (this is associated with the emergence of macrophage-focused direction of treatment of ischemic injury) .The structural recovery process begins at the end of the first week after two hours of ischemia. Therefore, there is morphological and functional evidence of ischemic and reperfusion injury of muscle tissue even one week after reperfusion. Delayed initiation of therapeutic

measures inhibits further regeneration processes and reduces the level of functional recovery of injured muscle tissue [27].

Ischemic skeletal muscle damage at the biochemical level is is a sequence of biochemical reactions that are initiated under conditions of hypoxia after a few minutes of ischemia and are the result of insufficient blood supply . Most cells die due to the activation of chemicals that are produced during and after ischemia and can be formed within a few days after normal blood flow is restored. After two hours of skeletal muscle ischemia and subsequent reperfusion, the ATP concentration decreases simultaneously with a significant increase in lactate content (from 25 to 114 mmol / kg dry weight). And after 3 hours of the process, the intramuscular supply of ATP is about 5% of baseline, and the glycogen pool is depleted by 88%. The enzyme creatine phosphokinase and myoglobin in serum and myoglobin in urine are the most characteristic biochemical compounds that are easily identified in ischemic clinical trials.

The following aspects can be identified by generalizing the biochemical pathways of pathological changes in ischemic skeletal muscle injury, which are dangerous for damaged tissue, as it loses 75% of myoglobin, 70% creatinine, 66% potassium and 75% phosphorus:

1. Lack of oxygen in the ischemic myocyte reduces the content of required ATP.

2. Myocytes are involved in anaerobic metabolism, producing lactic acid. The ion pumps fail due to a sharp change in intracellular pH and the cells become depolarized, which promotes the penetration of Ca^{2+} inside.

3. Ion pumps do not have the ability to cope with the transfer of Ca^{2+} from the cell and its intracellular content becomes too high.

4. Excess calcium ions leads to the generation of free radicals and calcium-dependent enzymes (calpain, endonucleases, ATPases and phospholipases);

5. The myocyte membrane becomes more permeable with excess phospholipases. More ions and pathogenic chemicals get inside the cell, mitochondria are destroyed, additional toxins and factors are produced.

6. After necrotic death of an ischemically damaged cell, toxic substances enter the intercellular space; the cascade of response to the inflammatory process is activated and phagocytic cells absorb such tissues.

7. There is the formation of tissue edema due to leakage of macromolecules (albumins) from damaged blood vessels due to their vasoconstriction [27- 31].

Carbon nanostructures - C60-fullerenes, which have unique physicochemical properties and biological activity are used in experiments to prevent, prevent and treat ischemic skeletal muscle injury [32]. Due to its spherical shape, small size (0.72 nm in diameter) and hydrophobic properties, the fullerene molecule is able to localize in the cell membrane and penetrate into the cells [33]. C60-fullerene interacts with free radicals and neutralizes them due to the presence of a conjugated system of double intercarbon bonds [34]. Also noted antiviral and antibacterial effects, effects on cell signaling systems, the activity of certain enzymes [35].

C60 fullerenes and their derivatives are able to inactivate oxygen free radicals. water-soluble C60-fullerene is a better antioxidant than natural vitamin E . One C60 molecule is able to capture 34 methyl radicals, as well as inactivate superoxide and peroxide anions both in vivo and in vitro. Chemical functionalization of C60-fullerene is accompanied by a decrease in the electron-acceptor and antioxidant properties of the framework. In vitro and in vivo studies have shown that these nanostructures and their derivatives do not show acute toxicity.

The use of fullerenes as powerful antioxidants is due to their ability to localize inside the cell in mitochondria and other organelles, which in pathological conditions is the formation of free radicals [36]. C60 fullerene and its derivatives are able to protect cells from various toxins.

C60-fullerene exhibits hepatoprotective activity: its aqueous suspensions, prepared without the use of polar organic solvents, are not only non-toxic, but also protect the liver from free radicals [37]. Antioxidant and antihistamine action of polyhydroxy derivatives of C60-fullerene has been established, which, according to the authors , is promising for use in the treatment of diseases such as asthma, polyarthritis, heart disease and multiple sclerosis.

One of the crucial roles is to maintain the innervating muscle of the nerve to effectively restore skeletal muscle function after ischemic injury.Studies indicate the effectiveness of C60-fullerene and its derivatives in combating inflammatory connective tissue replacement - fibrous degeneration of intervertebral discs, where the action of these nanoparticles is manifested not only in the absorption of free radicals, but also in a pronounced anti-inflammatory effect [38].

A very important indicator in studying the pathogenesis of ischemic muscle damage is the kinetics of fatigue caused by non-relaxation stimulation pools. Under normal conditions, its changes in the dynamics are reduced are detected after 5-6 hours of stimulation . Under conditions of ischemia, after 30-40 min it is not able to respond to the stimulation signal by generating force [39]. A linear decrease in the force response of the ischemic muscle was observed throughout the experiment. The protective effect of water-soluble fullerenes on the development of muscle fatigue at the level of 15 and 20-23% with its intravenous and intramuscular administration to rats at a dose of 1-1.5 mg / kg, respectively [40]. In the first variant of administration of C60-fullerene, the decrease in the force response was stopped and kept at the achieved level for two hours of non-relaxation stimulation. Changing the rate at which a force reaches its steady state during contraction is one of the most important indicators of the kinetics of skeletal muscle contraction [41]. This component of muscle dynamics is especially important in controlling accurate positioning. Ischemic damage to muscle tissue reduces this speed, which complicates, and sometimes completely blocks, the ability to accurately position the joint with such muscle. A biomechanical experiment to study the rate of achievement of the force of its steady state revealed a significant protective effect of C60 on the kinetics of contraction, intravenous administration of which maximized the rate of contraction by 25% compared to 15% of the protective effect of intramuscular injection. The effect of an aqueous solution of unmodified C60-fullerene on the dynamics of the development of the force response to stimulation muscle stimulation (muscle soleus) against the background of ischemic disorders that occur in the first 5 hours and within 5 days after 2 hours of ischemia and subsequent reperfusion was evaluated. The protective effect of the drug (compared with changes in the levels of force generation between the beginning and end

of stimulation pools) was 15% in the first 5 hours after ischemia and increased to 90% on the 5th day of the experiment. In this case, its intravenous administration was the most optimal - 92% protective effect with 63% for intramuscular injection.

1.4. Conclusions to the chapter

Therefore, it is possible to further use water-soluble derivatives of C60-fullerene (in the form of injection of their colloidal solution), taking into account their pronounced antioxidant properties and the lack of data on acute and chronic intoxication.

CHAPTER 2
QUANTUM-CHEMICAL DESIGN OF COMPLEX FORMATION OF THE "GUEST-HOST" TYPE OF C60 FULLERENES WITH LACTATES OF CERTAIN METALS

2.1.Methods of obtaining lactates

Lactate is often called lactic acid and vice versa. Let's clarify: lactate is a salt of lactic acid. Newly formed in skeletal muscle lactic acid is converted into lactate. From the muscles, lactate enters the blood, and from there - to the organs and other muscles, where it is used for energy production. In fact, lactic acid cannot accumulate in the body, lactate accumulates(Fig 2.1.).

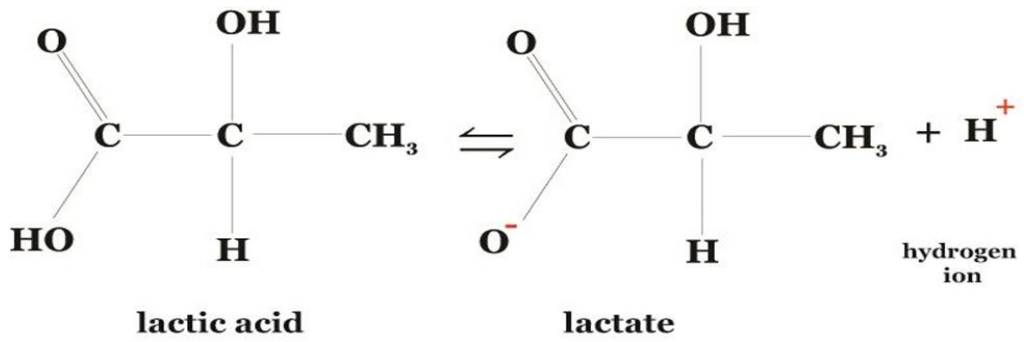

Fig 2.1.Lactic acid or lactate

Lactic acid is formed during the breakdown of carbohydrates - glycolysis. This is a complex chemical process with several reactions, but we will describe it more primitively. It is aerobic (involving oxygen) and anaerobic (without oxygen).

Aerobic glycolysis occurs when energy in the body is produced by the oxygen system. This is a normal life or light exercise of low and medium intensity. This glycolysis takes place in 2 stages:

Lactic acid and energy are formed from glucose and ATP molecules.

Lactic acid is neutralized by interaction with oxygen and ATP molecules, as a result of the reaction energy is released, carbon dioxide and water are formed.

As long as the oxygen system is working, lactic acid is not retained in the muscles .

Fast energy is required during intense loads. The oxygen system is slow, so the lactate power system comes to the rescue - it works without oxygen, does not spend time on its transportation, so it quickly produces energy. Glycolysis occurs: glucose is broken down into lactic acid and energy. Without the participation of oxygen, lactic acid is not neutralized, as in aerobic glycolysis, and accumulates in the muscles in the form of lactate.

Lactic acid CH3-CH (OH) COOH (α-oxypropionic, ethylidenemole) contains an asymmetric carbon atom and therefore may exist in optically isomeric forms.

Lactic acid can be obtained by various synthetic methods. Equal amounts of right and left isomers are always obtained. It is also observed in all other cases when substances containing an asymmetric carbon atom are obtained by synthetic reactions.

The reason for the mandatory formation of optically inactive compounds in synthetic reactions(Fig 2.2.) can be shown by the following examples:

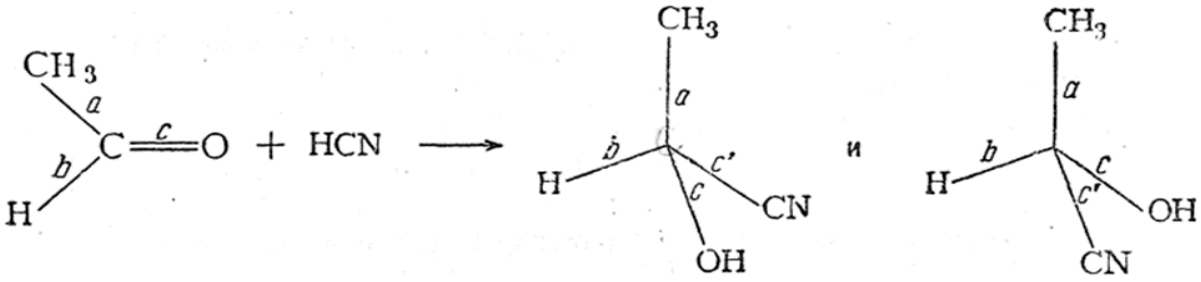

Fig.2.2. Synthetic reactions

As can be seen from the above scheme, under the action of hydrocyanic acid on acetaldehyde, the CN- anion can attack the π-bond of the carbonyl group equally likely on one side and on the other side of the plane in which the σ-bonds a, b and c ketone molecules. As a result, equal amounts of optically isomeric molecules of oxynitriles should be formed.

Similarly, in cases where an asymmetric carbon atom appears as a result of substitution reactions (Fig 2.3.)or cleavage reactions(Fig 2.4.).

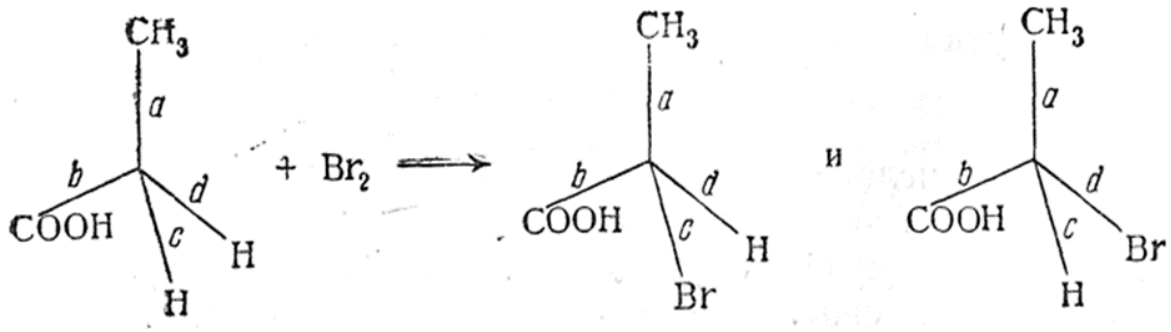

Fig 2.3. Substitution reactions

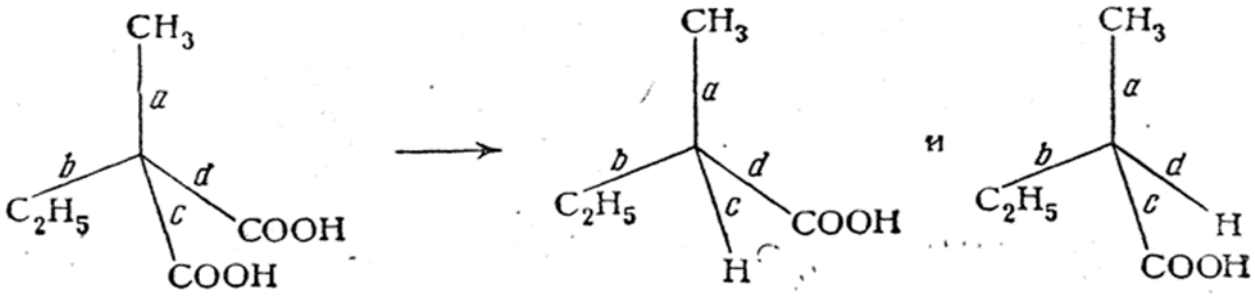

Fig .2.4. Cleavage reactions

The probabilities of formation of molecules of optical antipodes are absolutely identical, which should lead to the formation of optically inactive mixtures or racemic compounds .

Significant amounts of lactic acid are formed under the action of alkalis on aqueous solutions of the simplest sugars (monos). For example, a mixture of glucose and fructose ("invert" sugar) can produce up to 60% lactic acid. And in this case, inactive lactic acid is formed. [42].

The most important source of lactic acid is the process of lactic acid fermentation, which is easily subjected to solutions of many sugars (milk sugar, cane sugar, grape sugar, etc.). Fermentation is the result of the activity of lactic acid bacteria, the embryos of which are always in the air. The course of this process explains the presence of lactic acid in sour milk, from which it was first isolated Scheele, (1780). Lactic acid fermentation of sugar solutions is best carried out under the action of pure cultures of lactic acid bacteria (*Bacillus Delbrückii*) at a temperature of 34-45 ° C, with the addition of essential minerals for bacteria, as well as chalk or zinc carbonate. The latter additives are introduced to

neutralize the free acid, as at any significant concentration of acid bacteria die and fermentation stops.

Lactic fermentation is one of the processes that take place in the manufacture of butter (from sour milk), in the ripening of cheese, sauerkraut, in the ensiling of feed and the like. The equation of the lactic acid fermentation process has the form:

$$C_6H_{12}O_6 \rightarrow 2CH_3\text{-}CH(OH)\text{-}COOH$$

For lactic acid fermentation, as well as for alcohol, the existence of a special enzyme, zymase of lactic acid fermentation, which can cause fermentation without live bacteria (Buchner and Meisenheimer).

Usually lactic acid fermentation leads to the formation of optically inactive lactic acid, but often it turns out an acid with a weak right or left rotation.

Pure left-handed lactic (D-lactic) acid can be obtained by fermentation of sugars through a special fermentation agent (*Bacillus acidi laevolactici*). The right-handed isomer of lactic acid (L-lactic) was discovered by Liebig (1847) in meat extract and was named meat-lactic acid. Right-handed lactic acid is always found in the muscles of animals.

Ordinary (inactive) lactic acid, often called "lactic acid fermentation", has long been known only as a thick liquid. Careful corrective in high vacuum (0.1-0.5 mm Hg) can be obtained in the anhydrous state in the form of a crystalline mass that melts at 18 ° C. Of the salts of i-lactic acid is characterized by a well-crystallized zinc salt, which contains three molecules of water $(C_3H_5O_3)\ 2Zn \cdot 3H_2O$.

The difference between the properties of inactive lactic acid and optically active acids and their salts shows that the inactive substance is not a mixture, but racemic compounds of both (D- and L-) acids or their salts (lactates).

Right-rotating (L-lactic) and left-rotating (D-lactic) acids are airborne prisms with so pl. 25-26 ° C. They have a smooth but opposite optical rotation (in 10% solution [α] D15 ° C = ± 3.82 ° and in 2.5% [α] D15 ° C = ± 2,67°). With prolonged heating to 130-150 ° C optically active isomers are racemized and give anhydrides of inactive lactic acid. Zinc salts of optically active isomers of lactic acid crystallize with only two molecules of water $(C_3H_5O_3)\ 2Zn \cdot 2H_2O$ and both have exactly the same solubility in water (1: 175 at 15 ° C), different from the solubility of inactive salt (1: 50 at 10 ° C).

Optically inactive lactic acid can be separated into optically active isomers by molds, as well as by crystallization of lactic acid salts of optically active alkaloids: strychnine, quinine or morphine [43].

Lactic acid reactions are typical of α-hydroxy acids.

Especially easy (even when drying in vacuum) is the release of water with the conversion into lactide, which is a homologue of glycolide.

2.2. Closed aromatic surfaces

It is known that carbon has four allotropic modifications: diamond, graphite, carbine - well studied, and fullerenes (nanotubes, nanoring) - discovered less than three decades ago (Fig.2.1).

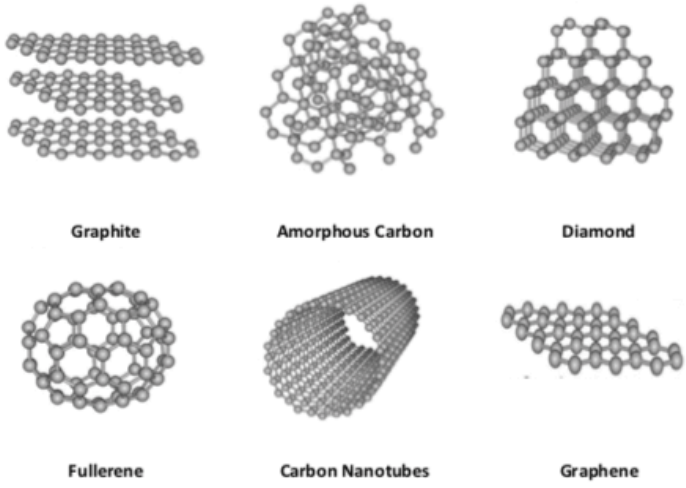

Fig.2.5. Allotropic modifications of carbon

The presence of sp2-hybridized carbon atoms made it possible to classify the latter as organic compounds. These structures did not appear to have functional groups such as, for example, -OH, -NH2, -COOH, etc., but their reactions were similar to those of aromatic compounds. Fullerenes, nanotubes and nanoring are characterized by addition reactions of, for example, free organic radicals, ē, H2, F2, etc. They have a non-planar structure, so they are called quasi-aromatic, ie those that are similar to aromatic.

Fullerenes, NT and NC have a closed 3D surface - hence the established term "closed aromatic surfaces" (ZAP).

The uniqueness of the physical properties of ZAP (in particular, fullerenes) has been of particular interest in recent years. Their specific band structure with a zero band gap and a linear dependence of the energy spectrum of electrons and holes on the wave vector leads to the fact that electric charges in them behave like relativistic particles with zero effective mass [44]. Anomalous transport and field effects open wide prospects for their practical use in nanoelectronics [45]. It is suggested that such nanostructures are promising materials of spintronics due to the significant length of the free path of electrons, the small value of the spin-orbit interaction, as well as the long spin scattering time [46]. Thus chemical or physical modification of ZAP gives the chance to reveal their new extraordinary properties. Thus, the complexation of fullerenes with other molecules allows to change the position of the Fermi level, the relative concentration of electrons and holes, without significantly changing the band structure of the original nanomaterials.

On the other hand, the unique biological properties of lactates stimulate the creation of complexes with fullerenes based on them, which opens the possibility of obtaining new therapeutic substances.

Since the establishment of the relationship "structure-property" is an important task of chemistry and physics of materials, the purpose of this work was to study the structure of complexes of fullerene C60 with lactates of some metals when heated by molecular dynamics MM+, semi-empirical quantum chemical PM3 and Monte-Carlo, calculate UV spectra of complexes depending on the concentration of lactates and determine the association constants of the systems.

2.3. Research model and methodology

The initial structure is fullerene C60 (60 carbon atoms). Complexation involves the sorption of lactates on the outer surface of fullerene.

As a ligand taken molecules of lactates of metals, namely: Mg^{2+} Ca^{2+}, Sr^{2+}, Zn^{2+} (Fig. 2.6.). Coordination by free p-orbitals of cations to the lateral surface of fullerene is

assumed. It is C60 that behaves like a strong ligand while having a powerful 60π-electron system with aromatic properties. It is their relative location, orientation relative to the fullerene walls, the quantitative characteristics of binding to C60 with changes in temperature that are the subject of the calculations.

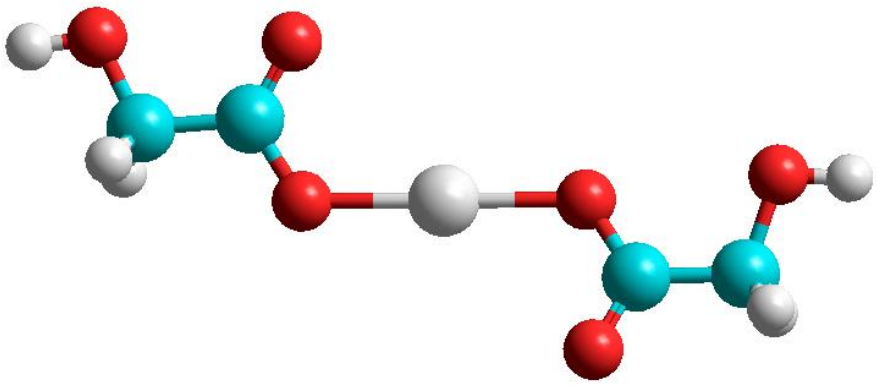

Fig. 2.6. Lactate molecule (Mg^{2+} Ca^{2+}, Sr^{2+}, Zn^{2+})

In the model under consideration, the interaction potential between Met^{2+} metal ions (see equation (1)) and the lactate anion (Met–O bond) was directly coupled with the pair potential of high energy perturbations of atoms [47-49] and was described by the Born-Mayer equation in within 0-0.75 nm of effective interaction radius for Mg^{2+}, 0-0.9 nm for Ca^{2+}, 0-1.00 nm for $Sr^{2+,}$ 0-0.675 nm Zn^{2+} (see equation (2))

$$U(r) = 4\varepsilon\left[\left(\frac{\sigma}{r}\right)^{12} - \left(\frac{\sigma}{r}\right)^{6}\right], \quad (1)$$

where r is the distance between the centers of the particles, ε is the depth of the potential well, σ is the distance at which the interaction energy is zero (the parameters ε and σ are the characteristics of the atoms of the corresponding substances);

$$U << E_0 \approx \frac{\hbar^2}{mr^2}, \quad (2)$$

where m is the mass of the particle, r is the distance between the interacting particles.

The Tersoff-Brenner interatomic interaction potential was used to describe the interaction of atoms at a distance of less than 0.2 nm . The total potential energy of the

system U is expressed as the sum of the binding energies of all pairs of atoms that make up this system

$$U = \sum_i \sum_{j>i} \left[V_R(r_{ij}) - B_{ij}^* V_R(r_{ij}) \right] \quad , (3)$$

where rij is the distance between the i-th and j-th atoms; VA (r) and VR (r) are exponential functions of the Morse potential type, which corresponds to the energies of attraction and repulsion between atoms; Bij * is a function that expresses the dependence of the binding energy of the atoms i and j on the angles θijk between the bond i-j and the neighboring bonds i-k and j-k.

To describe the interaction of atoms at a distance of more than 0.21 nm, we used the potential of the interatomic interaction of Tersoff-Brenner [50] together with the pair potential of Ziegler-Birzak-Litmark [51]. The lengths of the CC bonds in fullerene were 0.139 nm, and the Met – C interaction was described by the Leonard-Jones pair potential [48] with a potential interaction energy of 0.12 eV. The simulated period of one cascade of perturbations was 2 ps, and the law of conservation of energy in each calculation cycle was correlated within 0.15%. The initial coordinates of the intercalate were chosen according to the law of random numbers.

To solve the above problem, the following numerical simulation scheme was used: at the first stage of calculations the MM + method was used; on the second - semi-empirical PM3 method; and finally - the Monte-Carlo method. To calculate the association constants of the formed complex, we used a modified Beneshi-Hildenbrandt method [50], which takes into account the data on the maxima of fullerene absorption at different concentrations of lactate in the UV spectra.[52]

$$C_{quest}(A_0 - A) = \frac{1}{K_{ass} \cdot \Delta\varepsilon} \cdot \frac{1}{C_{host}} + \frac{1}{\Delta\varepsilon} \quad , (4)$$

where Cquest and Chost are the concentrations of “host” and “guest” respectively; A0 and A - absorption of fullerene C60 and "C60-lactate", respectively; Δε is the molar coefficient of extinction.

2.4. Results and discussion

As a result of the modeling of the fullerene-lactate system, the following facts were established: first, all lactates of the studied metals are characterized by similar behavior. Secondly, up to a temperature of ~ 550-600 K lactates are quite strong fullerene. However, in the case of gradual heating, two of the four lactate molecules (except strontium lactate) are desorbed when the temperature reaches as high as ~ 750 K (for SrLac2, this is only 650 K). The other two lactate molecules are in the zone of effective interaction radius and remain sorbed to temperatures of ~ 750 K, and in the case of higher values - only one lactate molecule remains (MgLac2, CaLac2, ZnLac2). Finally, the lactate is coordinated to the hexagonal ring of fullerene (and not to the Pentagon) by the coordination center - the metal cation.

The initial studied system, shown in Fig. 2.7. Turned out to be generally quite resistant to heat in a wide range of temperatures (up to ~ 800 K). The deformation oscillations of the fullerene crystal lattice do not exceed 0.015 nm, and the lactate molecules do not exceed 0.025 nm, which ensures the configurational and conformational stability of this system.

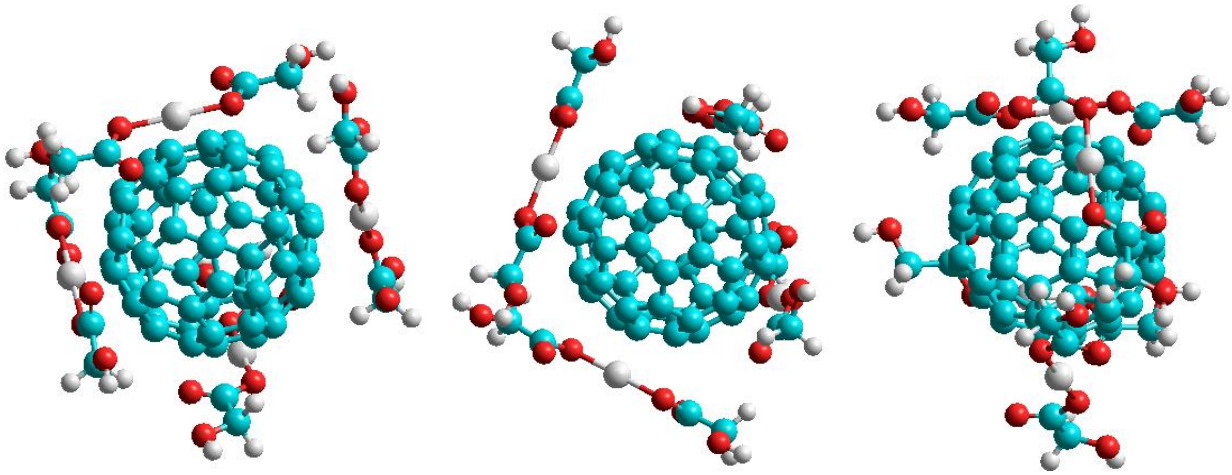

Fig. 2.7. Geometric model of the original studied system "guest-host" fullerene-lactate (orthogonal projections)

The dependence of the energy of the model system on temperature is shown in (Fig. 2.7.). As can be seen, at the initial heating from 273 to~ 550 K, the energy of the system

gradually increases, rises rapidly in the range of 600-750 K, and then with increasing temperature reaches the plateau, which indicates its high stability up to ~ 900 K.

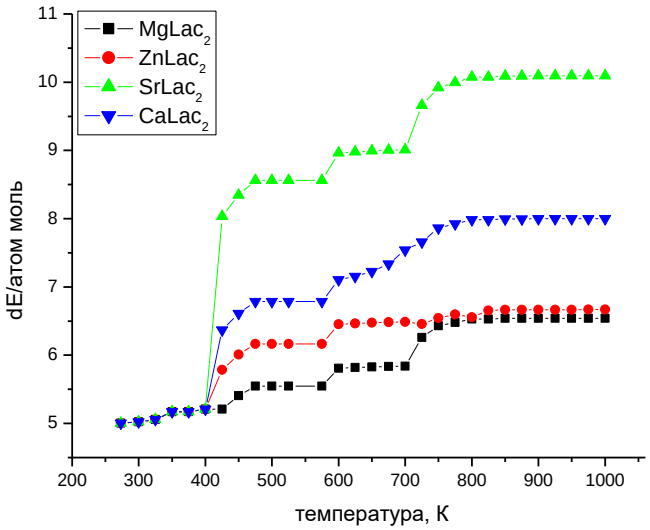

Fig. 2.8. Dependence of energy of the model system "fullerene-lactate" on temperature

As a result of the simulation, it is important to note the following dynamics of the structure of the complex during heating: at the initial heating to ~425 K the system remains quite stable, and the phenomena of extrusion of lactate molecules do not appear. There are oscillating and rotational (along the axis of the sixth order fullerene) possibilities of bonds and angles of C60 and lactate molecules; increasing the system temperature to ~600 K, there is a rapid elimination of half of the lactates from the outer surface of the fullerene. At temperatures above ~ 750 K there is almost complete external desorption of lactates, except for the last molecule (Fig. 2.9.).

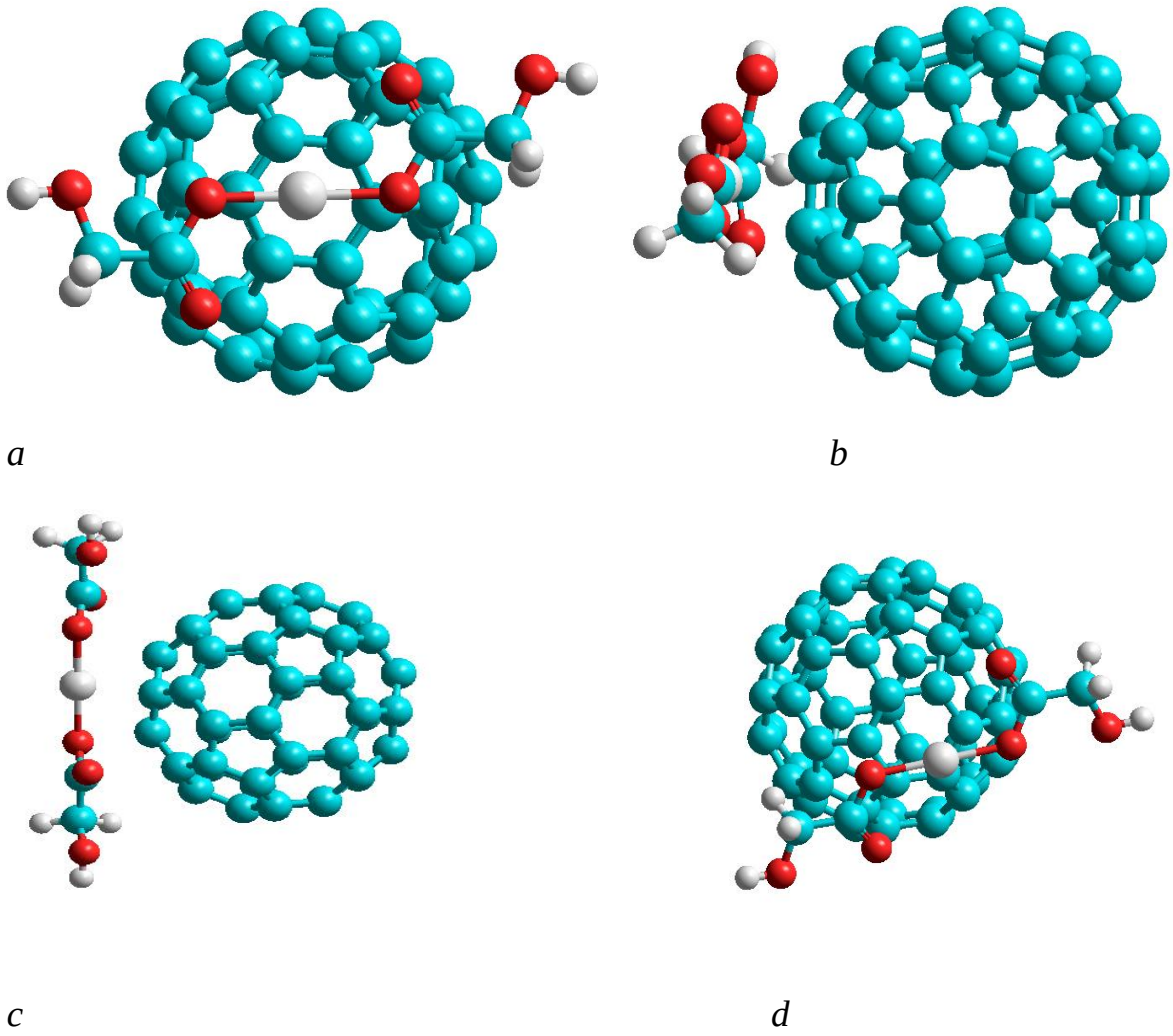

Fig. 2.9. Screenshot of the final structure of the system "C60 - lactate" when heated (*a*, *b*, *c* - orthogonal projections; *d* - side view)

It should be noted that in this version of the proposed model, the thermodynamic selectivity of physicochemical sorption-desorption is traced. In the temperature range 273-490 K there is a physical sorption, the nature of which is likely to overlap the unhybridized orbital 3dxy of Met^{2+} ions with the π-system of the lateral surface of fullerene, while chemisorption manifests itself at higher temperatures (~600 K), which is characteristic for π-π interactions of aromatic and quasi-aromatic cyclic and heterocyclic systems, which is observed in magnesium and zinc lactates.

There has been theoretically studied the "C60 - lactate" complex. The program varied the lactates ratio from 4 to 1 at constant concentration of C60 fullerenes. Optical

density was calculated at 198 nm, which is recommended in the reference literature for complexes with fullerene. The results of spectral calculation are given in (Table2.1).

Table 2.1

Calculated data of the "C60 - lactate" complex

Ratio of Complex components	Molar extinction coefficient, ε	Wavelength, λ (nm)	Δ
1:0	0,101	196	
1:1	0,112	197	0,011
1:2	0,125	198	0,024
1:3	0,134	199	0,033

Finally, the theoretical calculation of UV absorption spectra of fullerene depending on the concentration of lactates on the lateral surface in the modified Benes-Hildenbrand method (Fig. 2.6.) Shows that the association constants of the studied systems are 68.5 l • mol-1 (for CaLac2); 55.3 mol-1 (for SrLac2); 98.5 l • mol-1 (for MgLac2); 108.2 l • mol-1 (for ZnLac2).

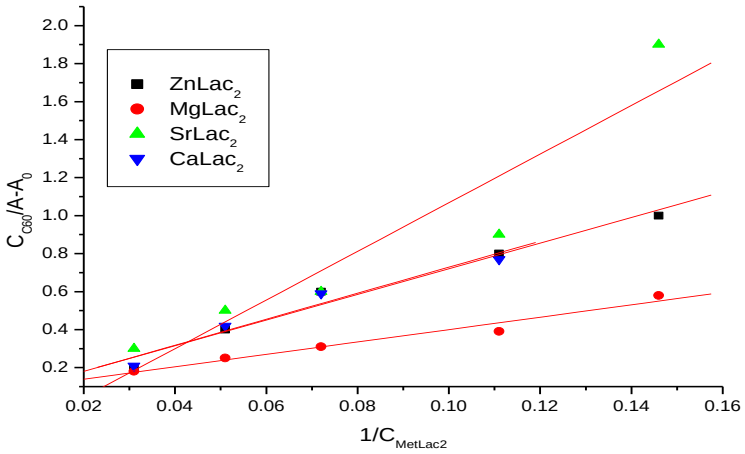

Fig. 2.10. Dependence of C60 absorption when lactate is added in Benesi-Hildenbrandt coordinates

2.5. Conclusions to the chapter

As a result of the calculations, it was found that the system "fullerene-lactate" is quite stable at elevated temperatures (up to ~ 550 K), which provides reliability and stability of the synthesis of complexes under normal conditions for this procedure. However, with subsequent heating, there is a gradual desorption of half of the lactate molecules and, finally, there is a thermodynamic stabilization of the system in the ratio of fullerene-lactate 1: 1. The system constants of systems are theoretically calculated.

CHAPTER 3
METHODS OF PRODUCING WATER SOLUTIONS FULLERENES AND COMPLEX IN WATER SOLUTIONS

3.1. Ultrasonic dispersion of organic solutions C60

The most well-known methods of obtaining aqueous dispersions of C60 are based on the transfer of an organic solution of C60 (in benzene, toluene or tetrahydrofuran) in the aqueous phase using ultrasonic treatment or prolonged stirring in clean water. Ultrasonic dispersion of an aqueous suspension is used to purify and separate fullerenes from each other and from the benzene phase [53].

Dispersants are devices for repeated grinding of particles in order to obtain a stable dispersed system. To do this, the ultrasonic dispersant first grinds the starting material using ultrasonic pulses or pulsations, and then the resulting particles are homogenized with a dissimilar medium. The laboratory dispersant is intended for work with small volumes of the investigated solutions. Ultrasonic dispersion is an example of the use of physical grinding methods. The dispersing effect of ultrasound is due to the fact that during the passage of the sound wave in the liquid there are local rapid compression and stretching, which lead to the dispersion of suspended particles.

Fig. 3.1. Ultrasonic dispersant

For this stage of the experiment, seven sterile falcon tubes of 50 ml are prepared. In the first: strontium lactate. Use a 10 ml solution with a concentration of 0.142 mol / l (C = 1.42 mol / l: 10 = 0.142 mol / l). Then add 9 ml of deionized water.

In the second: barium lactate. Take a 10 ml solution with a concentration of 0.125 mol / l (C = 1.25 mol / l: 10 = 0.125 mol / l). Then add 9 ml of deionized water to form 19 ml of solution.

Third: magnesium lactate. Since all other lactates are formed in the form of crystals, they must be diluted to a liquid. Mr (MgLac2) = 202 (this is one mole of the substance), and we need a 0.1 mmol solution (202/1000 = 0.2 g = 200 mg). After hanging, the real weight of magnesium lactate is 0.199 g = 199 mg. Then the crystals are poured into a glass beaker and 19 ml of deionized water is added. Since the crystals are well soluble in water with increasing temperature, a plate is used. Dissolution of magnesium lactate occurs rapidly.

Fourth: calcium lactate. Since all other lactates are formed in the form of crystals, they must be diluted to a liquid. Mr (CaLac2) = 218 (this is one mole of the substance), and we need a 0.1 mmol solution (218/1000 = 0.218 g = 218 mg). After hanging, the real weight of calcium lactate is 0.217 g = 217 mg. Then the crystals are poured into a glass beaker and 19 ml of deionized water is added. A plate is used to dissolve calcium lactate crystals. The reaction is fast.

Fifth: zinc lactate. Dilute lactate crystals to liquid with deionized water, because lactic acid with zinc oxide was diluted with water (water is better than methanol and isopropanol is used as a solvent). Mr (ZnLac2) = 243 (this is one mole of the substance), and we need a 0.1 mmol solution (243/1000 = 0.243 g = 243 mg). After hanging, the real weight of zinc lactate is 0.242 g = 242 mg. After that, the crystals are poured into a glass beaker and 19 ml of deionized water is added. A high temperature plate is used to dissolve zinc lactate crystals. The reaction is fast.

Sixth: zinc lactate is filtered. Dilute lactate crystals to liquid with deionized water, because lactic acid with zinc oxide was diluted with it (water is better than methanol and isopropanol is used as a solvent). Filtered zinc lactate is used in wet form. Mr (ZnLac2) = 243 (this is one mole of substance), and we need 0.1 mmol solution (243/1000 = 0.243 g =

243 mg), but we need to take more, because in the wet form there is a certain amount of water, which cannot be determined . After hanging, the weight of zinc lactate is 0.275 g = 275 mg. Then it is added to 19 ml of deionized water and under the action of temperature the precipitate gradually dissolves. The reaction is fast.

Seventh: 0.03 g (30 mg) of fullerene C60 and 15 ml of benzene (used as a solvent). A purple solution is formed in which the fullerene particles partially remain at the bottom of the falcon tube, so it requires long stirring until complete dissolution of fullerene C60 in benzene. After that, 15 ml of deionized water is fed and the aqueous and benzene phases are separated in a test tube. After that, the solution is subjected to ultrasonic dispersion of an aqueous suspension with a frequency of 45 kHz, an intensity of 50 W / cm² for 60 seconds. With a stop of 1 minute, the dispersion is repeated in the same way. The aqueous solution becomes cloudy. The intensity of fullerene with benzene decreased and went into the aqueous phase.

The resulting aqueous solution of fullerene is added to 1 ml in six falcovan tubes with lactates of strontium, barium, magnesium, calcium, zinc and filtered zinc.

Therefore, ultrasonic dispersion is an effective way to obtain aqueous dispersions C60, which are based on the transfer of organic solution into the aqueous phase using ultrasonic treatment. Benzene is used as a solvent.

3.2. Spectral studies of the C60 complex with magnesium lactate

The complex of magnesium lactate with C60 fullerene was studied experimentally. The concentration of the first varied from 3.52×10^{-4} to 1.76×10^{-3} mol • l^{-1} at a constant concentration of C60 3×10^{-5} (3.05×10^{-5}) mol • l^{-1} in water after ultrasonic dispersion. The optical density was measured ** at 200 nm, which is recommended in the literature for complexes with C_{60} fullerene. The results of the spectral study are shown in (Fig.3.2.).

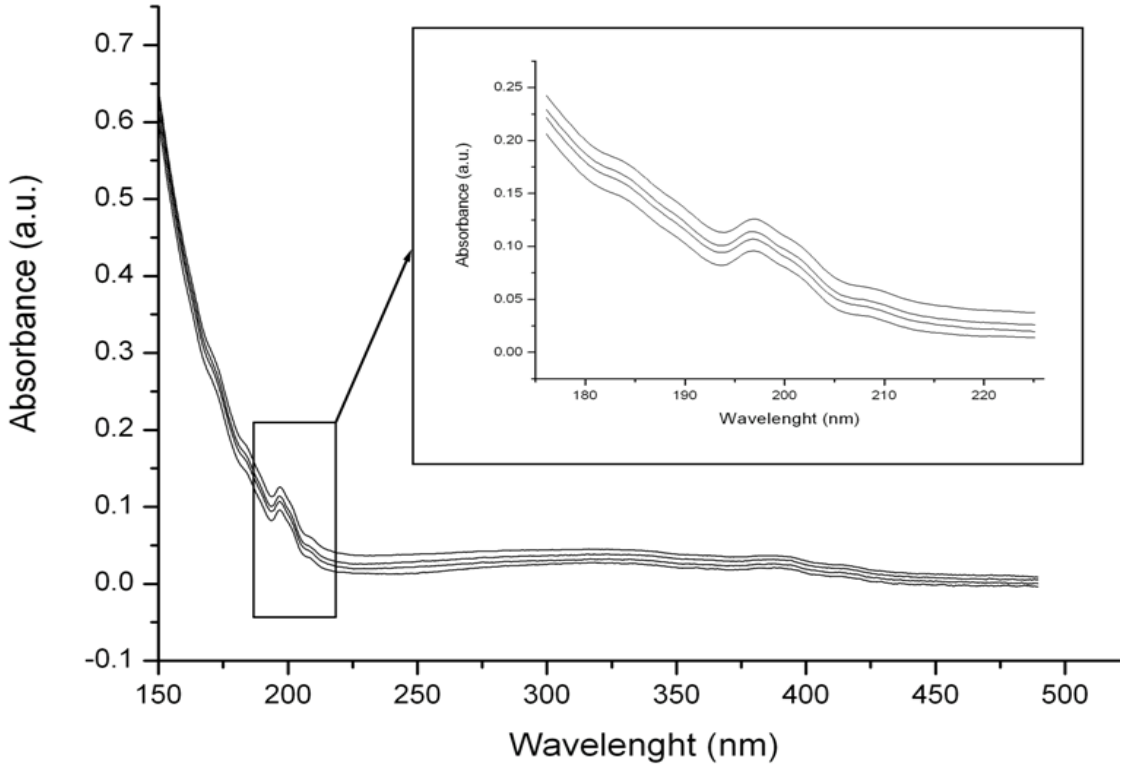

Fig. 3.2. Change in the electronic spectrum of fullerene C_{60} (in H_2O) when adding MgLac2

With such a ratio of components, there is theoretically the possibility of forming complexes such as 1: 1, 1: 2, 1: 3, and so on. However, the results of processing the spectra by the Beneshi-Hildebrandt method [54-57] indicate in favor of a structure of type 1: 1, and its stability constant is 108.2 l • mol^{-1} with an accuracy of calculations kkor ≥ 0.999.

Solutions of 5 ml of each concentration were prepared. Since the concentration of fullerene is constant, always take the same amount of the latter - 2.5 ml, and vary only the amount of solvent and magnesium lactate. The procedure for preparing solutions of known concentrations is given in (Table 3.1.). It also presents experimental data obtained by electron spectroscopy.

Table 3.1

Experimental data on the interaction of magnesium lactate with C60

V_{MgLac2}, ml	$V_{solvent}$, ml	C_{MgLac2}, moll·l^{-1}	C_{MgLac2} $C_{fullerene}$	Δ absorption
2,5	0	$1{,}76 \cdot 10^{-3}$	58,66	0,00278
2	0,5	$1{,}41 \cdot 10^{-3}$	47	0,00446
1,5	1	$1{,}06 \cdot 10^{-3}$	35,5	0,00644
1	1,5	$7{,}03 \cdot 10^{-4}$	23,4	0,0104
0,5	2	$3{,}52 \cdot 10^{-4}$	11,7	0,025

To calculate the association constant (stability) of the complex using a modified Benesi-Hildenbrandt method, which consists in plotting in the appropriate coordinates (Fig. 3.3).

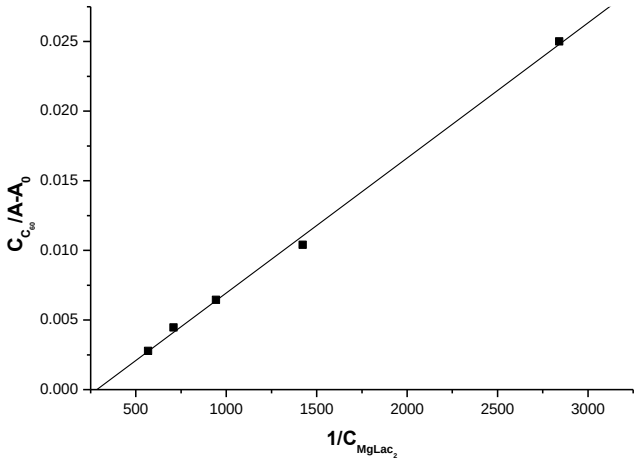

Fig. 3.3. Absorption of fullerene C60 when adding MgLac2 in Benesi-Hildenbrandt coordinates

Based on data on the absorption maxima of the complex or direct calculations by the formula:

$$C_{quest}(A_0 - A) = \frac{1}{K_{ass} \cdot \Delta\varepsilon} \cdot \frac{1}{C_{host}} + \frac{1}{\Delta\varepsilon} \quad (5)$$

where Cquest and Chost are the concentrations of “host” (C60) and “guest” (MgLac2), respectively; A0 and A - absorption of fullerene C60 and “C60-MgLac2”, respectively; $\Delta\varepsilon$ is the molar coefficient of extinction.

3.3. Conclusions to the chapter

The protective role of water-soluble derivatives of C60-fullerene in neurodegeneration and a significant increase in the tolerance of nervous tissue to hypoxia has been proved.

CONCLUSIONS

The thesis results in the design of bioavailable drugs for the correction of pathological conditions of traumatic origin, accelerating the recovery of the muscular system after motor dysfunction caused by these conditions, and reducing long-term pathological motor disorders. Metal lactates, fullerenes in aqueous suspension, metal lactate complexes with C60 fullerene and their influence on muscle activity are theoretically modeled.

The design provides:

1. The expressed protective effect of fullerene C60 on contractile dynamics of an ischemic injury of a soleus muscle;

2. The expressed protective effect is connected with generation of the maximum force of the answer caused by increase in level of muscular fatigue;

3. The protective role of water-soluble derivatives of C60-fullerene in neurodegeneration and a significant increase in the tolerance of nervous tissue to hypoxia has been proved;

4. The use of aqueous solutions of fullerene C60 and complexes with it, given the pronounced antioxidant properties and the lack of data on acute and chronic toxicity, opens new opportunities in the treatment and prevention of ischemic pathologies.

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