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HEALTH, BIOECOLOGY and NANOBIOSENSORS

Edited by Nadiya Skotna, Svitlana Voloshanska,
Taras Kavetskyy, Nataliia Stebeletska, Arnold Kiv

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This research work belongs to a group of authors, contains an in-depth study of the health preservation problem and the use of bioecology and nanobiosensors for this purpose, fixes the scientific priority, provides society with the primary scientific information on health promotion, the formation of environmental responsibility.

The monograph is intended primarily for scientists and meets by its content and form of publication, but will be interesting for a wide range of public. The clarity of the wording and presentation of the material, the logic of coverage for the basic ideas and concepts in it are of particular importance. Requirements to the essence of the presentation of the material in the sections of the monograph, similar to the requirements of other scientific publications with certain features of their purpose. Moreover, the issues raised in this monograph are still the subject of lively discussion among contemporary domestic and foreign scholars.

We will be glad if the monograph will not leave you indifferent and you will want to share your impressions of it.

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P R E F A C E

Currently, considerable attention is paid to the development of nanobiotechnologies in relation to the creation of new diagnostic and therapeutic methods in medicine. Important advances have already been made in the fields of pharmaceuticals, gene therapy, regenerative medicine and disease diagnosis. Nanobiotechnologies offer great opportunities in the diagnosis and prevention of COVID-19 through various approaches, including the development of infectious-safe personal protective equipment for medical workers and the development of active antiviral disinfectants, as well as the creationt of highly sensitive nanobiosensors for the rapid identification of viruses.

The nanobiotechnology developments increase the survival rate of cancer patients by enabling early decrease detection. Nanobiotechnlogical methods help in solving many problems in our life. They contribute to the degradation of waste and toxic pollutants. Bioremediation methods have proven operative in restoring environments with different types of pollutants.

The integration of different nanobiotechnologies is very promising in further development and production of biosensors. Nanosized materials have unique characteristics: a high sorption capacity, ability of self-assembly and, in some cases, unique catalytic properties. That is why nanobiosensors with a bioelement (biorecognition unit) and a physical transducer (signal converting unit) have been successfully used for enhancement of functional properties of the enzymatic sensors.

The main focus of the monograph is paied to concepts, and perspectives of practical applications of results obtained due to simultaneus contributions from different branges of chemistry, physics, biology, and materials science. The articles in this volume show the importance of interdisciplinary research that forms the backbone of the progress of the modern science.

The book is divided into two sections. The first one devotes to the role of nanobiotechnology to solve the tasks of practical medicine, in particular, health and bioecology. The second section is devoted to nanobiosensors.

We hope that the results of theoretical, methodological and practical problems considered in the collective monograph will be interesting both for specialists and for other categories of readers.

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SECTION I

HEALTH AND BIOECOLOGY

Chapter 1. RATIONALE FOR THE USE OF NON-TRADITIONAL TREATMENTS IN PATIENTS WITH BACK PAIN COMPLICATED BY DISC HERNIATION

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Abstract. *According to an epidemiological study of pain syndromes conducted in 19 European countries, 40% of out of more than 23,000 respondents reported back or neck pain and 21% reported foot or leg pain. In 2018 the Lancet Working Group on Low Back Pain identified a global problem of improper treatment of low back pain. For patients with low back pain, all six major international clinical guidelines preferred non-medical approaches to treatment. CareTrack study in 2019 showed that 28% of medical care for low back pain in Australia and the United States contradicted the clinical guidelines. Taking into account that with the compression of segmental structures there is a violation of venous outflow, paresis of small and big vessels, edema, sludge-shaped elements, microthrombosis, which also provoking decrease in the number of functioning capillaries; that is also true for the peripheral nervous system lesions caused by viral infections, especially coronavirus infection. In our work we during the we used non-traditional, pathogenetically determined methods of treatment in patients with back pain complicated by intervertebral disc herniation. We are considering it expedient to carry out conservative treatment by methods of manual therapy, reflexology (including acupuncture, Lyapko applicators, acupressure), ozone therapy, vacuum therapy, physiotherapy, in particular percutaneous electrical stimulation with acupuncture, kinesitherapy.*

Key words**:** *back pain, coronavirus infection, manual therapy, acupuncture, ozone therapy, percutaneous electrical stimulation, acupressure, vacuum therapy, Lyapko application devices, kinesitherapy*.

Introduction

In Ukraine, back pain (degenerative-dystrophic diseases of the spine) numbers vary up to 144.3 per 10k population, disability due to degenerative-dystrophic pathology of the spine - up to 3.1 per 10k population. The proportion of patients with degenerative-dystrophic diseases reaches more than 80% of all patients with spinal pathology and more than 30% in neurological departments (Babinets, & Nadkevich, 2019).

According to an epidemiological study of pain syndromes conducted in 19 European countries, out of more than 23,000 respondents, 40% reported back or neck pain, 21% reported foot or leg pain, and 22% suffered hand or arm pain (Todd, McNamara, & Balaj, 2019).

In recent years, the attention of not only doctors but also the population to non-traditional methods of treatment has increased both in Ukraine and abroad.

At the beginning of the XXI century, a written survey was conducted among 10,000 residents of the United States, Britain and Israel on the topic "Have you resorted to alternative medicine or non-drug treatments if you still haven’t recovered?". More than 55% (5646) of people gave a positive answer. The second question concerned which method the respondents gave greater preference. The answers were distributed as follows:

30% – used herbal medicine,
20% – used homeopathy,
15% – acupuncture and cauterization,
13% – used manual therapy,
8% – used hypnotherapy,
7% – meditation and relaxation,
2% – used the services of a masseur,
2% – used aromatherapy (Dovgyi, Sereda, & Khanenko, 2007).

A survey of nearly 200,000 people in 43 countries found that patients with back pain were almost twice as likely to have one in five mental health disorders (depression, anxiety, stress, psychosis and insomnia) compared to people without back pain (Mendelevich, 2019). Pain and depression are also independently associated with chronic fatigue syndrome. The prevalence of chronic fatigue syndrome reached up to 70% in patients with chronic low back pain (up to 40% according to our data). Similarly, patients with chronic fatigue syndrome report a higher intensity of pain, depressive symptoms and a higher risk of disability (Snekkevik, Eriksen, Tangen, Chalder, & Reme, 2014).

It is important to consider the risk factors that increase stress: hypoxia, hypothermia, overheating and noise. Musculoskeletal pain is more common during cold work. Symptoms increase with people in a cold environment for a longer period of time (Pienimäki, 2002; Pienimäki, Karppinen, Rintamäki, & Borodulin, 2012; Mannekens, 2016). That is, staying or working in the cold may increase the risk of back pain (Burström, Järvholm, Nilsson, & Wahlström, 2013). According to our data, cold lesions (acute or chronic hypothermia) of patients come across in 22-30% of cases. The calf muscle is considered the most vulnerable part of the leg, as it is often exposed to hypothermia, overexertion, radiating pain in vertebrogenic pathology, especially of discogenic origin. Hypothermia, which is not an etiological factor, causes circulatory disorders in the root area, and in pre-existing vertebrogenic pathology can cause exacerbation of discoradicular conflict (Dovgyi, 2016).

We currently have the assumption that local hypothermia of the muscles of the extremities may play an etiological role in the occurrence of pelvic pathology and lower back pain due to the physical transfer of cooler blood from the lower extremities to the pelvic plexus, where cold spasm can cause blood circulation to get disturbed, creating conditions for the pathology development (research is still being conducted).

CareTrack studies have shown that 28% of low back pain care in Australia (based on 164 patients) and in the United States (based on 489 patients) comes contrary to clinical guidelines (Traeger, Buchbinder, & Elshaug, 2019). In 2018, the Lancet Working Group on Low Back Pain identified the global problem of improper treatment of low back pain. In particular, for patients with low back pain, all six major international clinical guidelines have given priority to non-medical approaches to treatment (Qaseem, Wilt, & McLean, 2017; Chenot, Greitemann, & Kladny, 2017; Stochkendahl, Kjaer, & Hartvigsen, 2018). Decreasing role of medical treatment for low back pain is reflected in recent clinical guidelines (Hartvigsen, & Hancock, 2018; Foster, Anema, Buchbinder R, & v. Tulder, 2018). For patients at risk of developing chronic pain and disability, experts are considering the possibility of offering procedures such as spine manipulation, massage, acupuncture, yoga, psychological therapy or multidisciplinary rehabilitation (Traeger, Buchbinder, & Elshaug, 2019).

It’s essential to remember that the peripheral nervous system is one integral system with the central nervous system (CNS) and interacts closely with it. It connects the CNS with all organs and tissues of the body, not only by conducting nerve impulses, but also by transferring macromolecules

between the CNS and internal organs. The amount of intracellular potassium and sodium, which balance is maintained by the sodium pump, determines the electrical activity of cell membranes.

The electrical activity of cell membranes is regulated by opening or closing the potential-dependent sodium and potassium channels located near the Ranvier constrictions. Axon's greatest energy needs are concetrated in the Ranvier constriction zone, where glucose is broken down aerobically (Dovgyi, 2016). This once again confirms the feasibility of acupuncture, percutaneous electrical nerve stimulation (PENS) and ozone therapy.

Myelin does not cover the nerve fiber completely, but intermittently at regular intervals (Ranvier interceptions). Between these gaps in the myelin sheath lay small gaps – so-called Schmidt-Lanterman notches. These are places where the formation of myelin cytoplasm is delayed due to circular twisting of the Schwann cell around the nerve fiber (Macheret, Dovgyi, & Korkushko, 2006). Actually Schwann cells make up a myelin cover and have length from 100 microns to 1 mm (Silantyev, 2006). In the case of microcirculation insufficiency, there are violations of venous outflow and increased pressure, in particular in the spinal plexuses (Dovgyi, 2016).

In general, the conduction of impulses is based on the electrical properties of excitability and conductivity, which are inherent in all electroexcitable tissues: nerve, muscle and glandular tissue. This is also one of the main functions of the neuron. Its membrane has an electric charge with a negative potential (more precisely, a resting potential) on the inner surface and a positive one on the outer surface. The resting potential, actively maintained by the cell, is from 50 to 90 mV. The potential is formed due to the redistribution of sodium and potassium ions. The cytoplasm of the neuron (compared to extracellular fluid) contains 10 times less sodium ions, up to 50 times more potassium ions and 50 times less chlorine ions. Irritation of the membrane by stimuli (chemical, mechanical, electrical) causes a change in ion concentrations: significantly increases the permeability of the membrane for the passage of sodium ions into the cell and to a lesser extent – for the flow of potassium ions in the opposite direction. In this process, the membrane depolarizes and the action potential is formed. The development of action potential occurs on the all-or-none principle (Belyakov, & Gustov, 2007).

The diameter of nerve fibers varies from 1.0-2.0 to 8.0-20.0 μm, depending on the degree of their myelination. Most peripheral nerves perform mixed functions, so they are composed of motor, sensory and autonomic fibers close together (Macheret, Dovgyi, & Korkushko, 2006). This explains the simultaneous occurrence of sensitive, motor and autonomic manifestations in case of nerve damage. However, more or less isolated disorders can occur with a selective lesion of a particular type of fiber.

Spinal nerves and their roots, compared to peripheral nerves, are covered with thin epineurium and perineurium with limited resistance to chemical and dyscirculatory stimuli. Each nerve fiber of the spinal nerve, in turn, is "dressed" in the endoneurium, the tubules of which contain Schwann cells covering the myelin sheath of the fiber.

Thin epi- and perineuria, poorly developed connective tissue structures of the spinal canal, are an unreliable barrier to maintain the ionic balance of interstitial fluid. These biochemical abnormalities complement the mechanical factor of nerve and root damage. As a result, this set of mechanical, chemical and dyscirculatory factors can lead to radicular lesions – radiculopathies (Ulysses, 2014).

Nerves are provided with vessels with a dense network of anastomoses. The circulatory and lymphatic systems are quite important for the nervous system.

Peripheral nerves contain sensitive and motor fibers, sensitive only fibers or motor only fibers. Sensitive fibers begin with the receptors on the skin, muscles, ligaments and bones, and end at the neurons of the posterior roots. When the sensitive nerves are damaged, the sensitivity changes within the innervation zone of the peripheral nerves (decreased skin sensitivity), hyperesthesia (increased sensitivity), paresthesia (unpleasant sensations of numbness), hyperpathy (distorted sensitivity with an increased threshold of its perception). Some sensitive fibers have a myelin sheath, some don’t. As a rule, motor fibers have a developed myelin sheath. When motor fibers gets

damaged, there are signs of weakness and decreased tone of certain muscles, their atrophy (with severe damage to axons). These injuries are manifested in motor disorders: fasciculations (short-term involuntary muscle contraction in the form of subcutaneous tremor), cramps (painful cramps), pseudoathetoid movements (slow involuntary violent movements due to tonic muscle contraction) and tremor (Silantiev, 2006).

Nerve dysfunction can be associated with: direct nerve damage or ischemia due to damage to its arteries (Dovgyi, 2016).

The spine is a kind of mobile "case" for the spinal cord and its roots. In the course of human life, degenerative-dystrophic changes develop in the spine structures: deforming spondylosis of the spinal longitudinal ligaments, deforming spondyloarthritis, osteochondrosis, disc fibrosis, vertebral osteoporosis, etc. At excessive loadings (or without them) on a backbone, in particular on intervertebral apertures, hernial protrusions of intervertebral disks, and is more exact extrusion, prolapse, and also protrusions, kernels of intervertebral disks can be formed.

An important point to be noted at once is that most common degenerative-dystrophic manifestations (protrusions and hernias of intervertebral discs) occur in the population of developed countries, where hypodynamia is a common finding. Asymptomatic intervertebral disc herniation is many times more common than intervertebral hernias, which cause back pain, or rather back and limb pain. Size does not matter much here (Sviridova, 2015).

Nerve roots pass through the intervertebral foramina slightly obliquely from top to bottom. In front of the roots the vertebral body is flowing into the intervertebral foramina; after leaving the intervertebral foramen, the root comes into contact with the posterolateral surface of the intervertebral disc. The nerve root, passing through the intervertebral foramen, occupies a third of the total volume. The rest of the hole is occupied by blood vessels, connective tissue, receptor nerves of the spine and spinal nerve. Thus, the intervertebral foramen is filled with following: nerve root, sinuvertebral nerve, artery, root vein, intervertebral veins. Actually, first of all, various mechanical or chemical processes have their influence on the veins (Fig. 1). Therefore, we believe that the appropriate tactics of pathogenetic treatment shall take into account these data (Dovgyi, 2016).

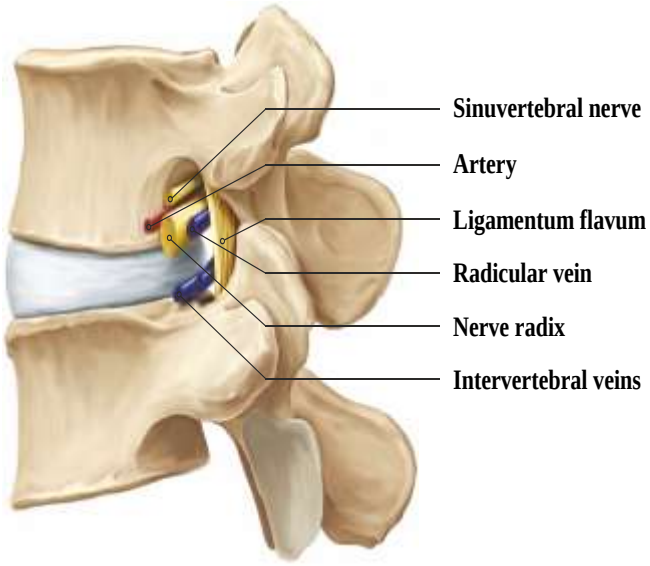

Fig. 1. Location of veins, arteries, nerves in the intervertebral foramen (Dovgyi, 2016)

The spine has at least four functions: supporting, protective, cushioning and motor. It is a flexible rod: a support for the head, shoulder girdle and arms, thoracic and abdominal cavities, the weight of which is transferred to the pelvic girdle and legs. Due to the supporting function, the vertebrae own a different structure. The protective function of the spinal cord is to protect the spinal

cord from mechanical damage. Essentially, the flexibility of the spine stays of great importance for the absorption of shocks and concussions, protecting the brain from trauma to the bone structure of the skull. Muscles, intervertebral discs, joint fissures, and vertebral joint surfaces are involved in cushioning. The presence of physiological curvature (cervical and lumbar lordosis) also plays a significant role here. Motor function is performed in the intervertebral joints around three axes: frontal, sagittal and vertical. There are passive part (vertebrae, joints, ligaments, discs) and active – the muscular system (Dovgyi, 2016). Muscles have vessels (arteries, veins) that can contract due to prolonged spasm of the same muscles. Prolonged vascular compression creates the conditions for the development of hypoxia in the so-called transitional areas with fewer functioning capillaries and, accordingly, poorer collateral blood flow (Kolisnyk, 2019).

We live in a three-dimensional world in which it is quite difficult to find motion occuring in one plane only. In wildlife it is almost impossible. And the vertebra therefore can and do move in all three planes.

The largest number of joints is located in the spine, reaching about a hundred. Former WHO Director-General G. Brundtland (doctor, former Prime Minister of Norway) said that diseases of the bones and joints make the main cause of persistent pain and physical deterioration.

Joints provide mobility of the musculoskeletal system. Functionally and clinically, joints are inseparable from muscles, ligaments, and the nervous system that controls movement. 70% of the information conducted to the brain is analyzed at the lower levels of the nervous system and only 30% reaches the cerebral cortex. At the same time, every muscle, even the smallest, owns its representation in the cerebral cortex (Sitel, 2008; Dovgyi, 2016).

The pathology of the spine and changes in the segments lead to dysfunction of the relevant organs, which are connected through the autonomic structures. The nature of microhemodynamic changes depends on the type of pathological autonomic impulse from the segments. Irritation of segmental sympathetic vegetative structures leads to excessive impulses, which causes spasms of arterioles and lower number of functioning capillaries. With a decrease in the intensity of autonomic impulses or impulses' blockade during compression of segmental structures paresis of microvessels, edema, sludge of shaped elements, microthrombosis occures, which also drops the number of functioning capillaries (Kolisnyk, 2002; Kolisnyk, 2019). It is important that the veins collect blood from the capillary bed through the anastomosing postcapillary venules. Increased vascular permeability is characteristic of inflammation (edema), observed in postcapillary venules (Kumar, 2019).

With the development of pathological changes in the shape of the spine the preconditions for dystopia, ie displacement of internal organs take place, leading to deformation of arteries, veins, lymphatic vessels involved in blood and lymph supply of organs, as well as excretory ducts, causing disruption of arterial, and most importantly venous and lymphatic outflow, violation of the evacuation of secretions (urine, bile, pancreatic juice etc.) (Kolisnyk, 2019).

The human spine goes through an individual path of development, which depends on certain living conditions. The concept of "living conditions" includes physical, psychological and emotional stress, disease, food quality, environmental conditions. And the "environmental conditions" that dramatically affect the development of the spine: the influence of gravitational forces, changes in atmospheric pressure, electromagnetic balance, thermal regime, ionizing radiation, and others. Transformations of the morphology of the skeleton and spine usually occur with an interval of 7 years (Tonkov, 1946; Orel, 2010).

The human body has a separate, as yet insufficiently studied, functional system of regulation of electromagnetic balance (system of electromagnetic homeostasis). It controls all the vital processes, ensures optimal performance of vital functions through close interaction with other known control systems (nervous, endocrine, immune). The system of electromagnetic homeostasis is represented by the corresponding structures at purely molecular, cellular, tissue levels. It is believed that the role of electromagnetoreceptors at the molecular and cellular level is performed by water and collagen molecules, and at the tissue level with the acupuncture points and the channel-meridian system (Golub, Koptelov, & Bondar, 2018). We are studying the system of

electromagnetic homeostasis using a device for monitoring the spectral-dynamic parameters (KSD), in particular for acupuncture diagnosis and subsequently for more effective treatment.

As an result, the spine of each person (although, perhaps, relatively) can be called unique. This should be considered when examining the patient, for examination, differential diagnosis and subsequent treatment tactics.

Analysis of recent research

First of all, patients with discogenic lesions of the nervous system in the acute period should be recommended for the appointment of the most gentle motor and physical regime to stay in a semi-hard bed. The duration of bed rest is not more than 2-3 days. After that, it is advised to gradually expand motor activity and therapeutic exercises to reduce the likelihood of developing chronic pain.

General principles of drug therapy of vertebrogenic pathology are aimed at the purchase or control of pain – nonsteroidal anti-inflammatory drugs, analgesics, antidepressants, improving regional blood and lymph circulation, microcirculation and tissue trophism – euphylline, nicotinic acid, No-spa, vectocontal, trentonal, trentonal euphyllin, detralex, as well as lasix, vitamins B, C, E, biogenic stimulants, including plasmol (Klimenko, Golovchenko, & Kalischuk-Slobodin, 2008; Dovgyi, 2016; Kozolkin, Medvedkova, & Revenko, 2020).

To eliminate the pain syndrome, various methods of reflexology are widely used: acupuncture, electrocupuncture, especially acupressure, micro-acupuncture, auriculopuncture, stimulation with a beam of needles. It is necessary to take into account the genesis of pain syndromes, the topic of the lesion (including muscular-tonic, vegetative-vascular and neurodystrophic manifestations, pathogenetic features of the pain syndrome, course and stage of the disease, degree of functional disorders), number of trigger points, presence of visceral pathology. when acupuncture is rather effective (Dovgyi, 2016; Kozolkin, Medvedkova, & Revenko, 2020). Superficial multi-needle therapy use Lyapko applicator devices (Lyapko, Dzhuzha, & Lyapko-Arshinova, 2009; Dovgyi, 2016).

Manual therapy (MT) is used to eliminate functional disorders of joints and muscles and to treat pain syndrome. For the treatment of vertebrogenic pathology of discogenic genesis, following classical MT methods are used: postisometric relaxation, mobilization, manipulation (Dovgyi, 2016; Traeger, Buchbinder, & Elshaug, 2019; Kozolkin, Medvedkova, & Revenko, 2020; Taran, 2021).

Ozone therapy is used to restore microcirculation and improve tissue trophism. In addition to the vasodilating effect, ozone therapy improves tissue oxygen supply, reduces tissue hypoxia, including tumor processes, activates gas exchange in the area of ischemia, improves peripheral blood circulation, facilitates venous outflow from the extremities, reduces tissue edema, 2003; Shmakova, Nazarov, Barkhotkina, & Ivanov, 2014; Dovgyi, 2016; Andryushchenko, 2019; Schwartz A, 2021). This is quite relevant given the postcocious syndrome, in particular the effects on blood vessels, especially veins, and increased immunity.

Among physiotherapeutic methods we prefer phonophoresis or electrophoresis with solutions of analgesic drugs, sinusoidal modulated currents, pulse currents of low frequency, in particular amplipulse therapy, diadynamic current; quite effective percutaneous electrical nerve stimulation (PENS). The complex of physiotherapeutic treatment also includes magnetic therapy, which performs the function of the basic method of long-term action. Drug medical blockades (DMB) are used to treat pain syndrome (with ozonated air or anesthetic), ie carrying the substance to the site of the pathological focus causing the pain (Dovgyi, 2016).

We also carry out kinesitherapy in order to strengthen the "muscular corset", following the main rules, including increasing the dose of exercise during the procedure and course (with a maximum load in the middle of the procedure) with repetition of each exercise 4-6 times (Kozolkin, Medvedkova, & Revenko, 2020).

Also, in addition to the above methods of treatment, patients at risk of developing chronic pain and disability, domestic and foreign clinicians, depending on what clinical recommendations

they have followed, consider offering procedures such as massage, yoga, psychological therapy or multidisciplinary rehabilitation (Dovgyi, 2016; Traeger, Buchbinder, & Elshaug, 2019).

Various methods of reflexology are widely used to eliminate back pain: acupuncture, especially in combination with PENS, micro-acupuncture, auriculopuncture, irritation with a beam of needles (Lyapko rollers), especially acupressure. Considering the pathogenetic genesis of pain vertebrogenic syndromes, the topic of lesions (local and distant manifestations, the presence of vertebral syndrome, muscular-tonic, vascular and neurodystrophic manifestations, pathogenetic features of pain, course and stage of disease, degree of pathological presence, degree of pathology) stays essential. (Dovgyi, 2016; Sviridova, & Morozova, 2017). Superficial multi-needle therapy uses Lyapko applicator devices (Lyapko, Dzhuzha, & Lyapko-Arshinova, 2009; Dovgyi, 2016).

One of the reasons for carring out kinesitherapy is to strengthen the "muscular corset", following the main rules, as increasing the dose of exercise during the procedure and course (with the maximum load in the middle of the procedure) with repetition of each exercise 4-6 times (Kozolkin, Medvedkova, & Revenko, 2020).

The purpose of the study: to use clinical-neurological, instrumental, laboratory methods to improve the understanding of the state of functional activity of the vascular system in vertebrogenic pathology, and to determine approaches to improving the algorithm of treatment of patients. We use magnetic resonance imaging (MRI), radiography with functional tests, capillaroscopy, duplex ultrasonographic scanning of arteries and veins of the lower extremities, device controlling spectral and dynamic characteristics of a human being (KSD), tests (visual-analog scale (VAS), McGill pain questionnaire, DN4 questionnaire, Hamilton depression rating scale) to clarify the pathogenetic mechanisms of the disease.

Material and methods of the research

On the basis of the Department of Neurology and Reflexology of the National University of Health of Ukraine named after P.L. Shupyk ("Doctor Dovgyi Clinic") we examined and treated 28 patients, 16 female (57.2%), 12 male (42.8%), age range 20 to 68 years. Duration of exacerbation of the disease from 1 week to 6 months.

The diagnosis was confirmed by MRI, lumbosacral radiography in two projections with functional tests, capillaroscopy, duplex ultrasonographic scanning of the vessels of the lower extremities, SDP, appropriate tests.

The patient's age, leading pathogenetic genesis of pain vertebrogenic syndromes, variants of the clinical picture of the neurological syndrome, somatic status, duration and stage of the disease are important in the treatment.

Results of the research and their discussion

Individual, consistent, pathogenetically conditioned treatment by methods of manual therapy, reflexology, including acupressure, vacuum therapy, physiotherapy, kinesitherapy and other non-drug methods was used.

MT techniques were chosen taking into account the characteristics of symptoms, including neurological, the severity of pain, the degree of tension in the muscles, the location of the hernia or protrusion of the intervertebral disc.

Before performing MT, segmental or acupressure massage was performed to relieve muscle spasm and reduce their tension. Acupressure has been performed in patients with radicular syndromes in combination with the Booster vibrating massager, especially to relieve gluteal hypertension on the affected side, which clinicians do not always pay attention to.

The MT session began with post-isometric relaxation techniques aimed at relaxing the muscles.

The success of manual treatment does not depend on the size of the hernia (including sequestered), but rather on the size of the vertebral canal, the condition of the muscular and especially the circulatory system, in particular the condition of the veins. This is even more relevant given the post-COVID syndrome.

We used classical acupuncture in the first one or two sessions used tonic acupuncture points (AP) and local points, pain points. Subsequently, the acupuncture procedure was selected

individually, taking into account the patient's condition: both local-segmental and remote AP were used simultaneously. The most commonly used AP on the meridian of the bladder V 22–28, 31–34, 46–50, 60–67; as well as T 3-5, located in the zone of innervation of the respective roots.

Lyapko applicator devices were used locally in the lumbosacral region and partially in the lower thoracic region. Needle elastic Lyapko bands used in patients with pain in the leg during the innervation of the nerve, wrapping them around the corresponding lower limb; course of treatment included 4-8 and more procedures. Also 4 (14.3%) patients who had post coronavirus syndrome used Lyapko applicator suit, during the use of which the body creates a positive training stress to increase immunity.

Ozone therapy was administered as intravenous injections 8 times a day, in case of severe pain we used the paravertebral blockade with ozonated air. The purpose of ozone therapy is to restore microcirculation and improve tissue trophism.

Kinesiotherapy was usually prescribed after 3-5 sessions, taking into account the pain syndrome, mobility in the vertebromotor segment, the state of the muscular system, weather conditions.

Vacuum therapy and jar massage with ozonated oil were used topically in the lumbosacral region and, in the case of radicular syndrome, along the corresponding root to improve venous outflow, local blood and lymph circulation.

If necessary, hirudotherapy was used to improve microcirculatory disorders, eliminate ischemia and tissue hypoxia, spasm of deep paravertebral muscles, as well as restore cerebrospinal fluid dynamics, treat aseptic inflammation.

8 (28.5%) patients were offered to wear a medium fixation corset with six ribs for 3-4 months to ensure the unloading of the affected spine.

Patients were offered infusions and decoctions used for pain in the lumbar spine, as infusion of horse chestnut, burdock, Adam's root, lilac, fresh lovage shoots, decoction of birch buds, burdock compress, grated horseradish with potatoes or radish.

Aromatherapy was used with playing melodies of calm rhythm. Various essential oils were used, including lavender, mint, rosemary, eucalyptus and pine.

Evaluation of treatment results was performed according to the regression of leading clinical symptoms (elimination of pain and sensory disorders, recovery of the muscular system), assessment of clinical and neurological status, MRI data, capillaroscopy, duplex ultrasonographic scanning of lower extremities, KSD, tests (visual-analog scale (VAS), McGill's pain questionnaire, DN4 questionnaire, Hamilton's depression scale).

Significant improvement was noted in 10 (35.7%) patients, improvement in 12 (42.8%), slight improvement in 4 (14.3%) and no improvement in 2 (7.1%) (Fig. 2).

Fig. 2. State dynamics

Conclusion

We analyzed use of non-traditional methods of treatment in patients with back pain complicated by hernias, their pathogenesis, conservative treatment. We consider it expedient to carry out conservative treatment individually, pathogenetically due to the methods of manual therapy, reflexology, including acupressure, ozone therapy, vacuum therapy, physiotherapy, kinesitherapy.

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Chapter 2. INFLUENCE OF CHROME CITRATE NANOPARTICLES ON GROWTH AND PROLIFERATIVE ACTIVITY OF *ALLIUM CEPA L.* AS A TEST-OBJECT

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Abstract. *The biological influence of the different chrome citrate nanoparticles (CCNP) concentrations on growth and proliferative activity of Allium cepa L. as test-object are investigated. It was found that aqua CCNP solutions in the range at concentrations of 0.25-2.50 mg/l do not cause phytotoxic effects: do not change the turgescence, color and shape of the test object bulbs roots; growth activity of its roots is at the level of control, and phytotolerance is high (94.64-98.57%). CCNP at concentrations of 5 mg/l-7.5 mg/l and above cause phytotoxic effects of high and maximum levels. It is shown that CCNP in the range at concentrations of 0.25-2.50 mg/l do not have a cytotoxic and mitosis modifying effect on the proliferative activity of cells apical meristem of the test object roots: mitotic and phase indices are within normal limits; indices of cytotoxicity are within background values. The Allium-test confirm the possibility of using CCNP in technologies of growing crops in the range of biologically acceptable concentrations (0.01-0.05 mg/l), which do not reveal any manifestations of phyto-, mito- and cytotoxicity*.

Keywords**:** *chrome citrate nanoparticles, phytotoxicity, cytotoxicity, Allium cepa L.*

Introduction

Intensive research is conducted in recent years in nanotechnology, nanomedicine, nanothermodynamics, nanoelectronics, etc. to obtain new, more efficient, and environmentally friendly nanocompounds and nanopreparations based on them. All this became possible due to the development of new technologies to synthesize substances in the nanoscale range (Viana, 2014; Huynh, 2020).

Different types of nanoparticles have already synthesized using nanotechnology methods (Viana, 2014). Among them, essential biometals nanocitrates (EBNC), which are a product of the synthesis of new technologies of Ukrainian researchers, obtained by the erosion-explosive method, which is based on a unique physical effect in the field of high energy concentration (Kosinov, 2009). The developers of this method of nanoparticles synthesis are positioned EBNC as a source of biologically available elements important for the plants (Huliaieva, 2018) and animal biogenesis (Zakharchenko, 2017).

It is believed that nanoparticles of citrates of essential elements are environmentally safe, non-toxic, biologically susceptible and functionally bioactive. In the form of carboxylates (citrates) of organic acids, when entering the cells directly enter the Krebs cycle - one of the leading energy exchanges of cells and organisms (Kosinov, 2009; Zakharchenko, 2017; Huliaieva, 2018). It is proposed to use environmentally friendly complex micro fertilizer “Super-Eco” in crop production and horticulture (Kosinov, 2010), which contains carboxylates of trace elements in specific quantities, including nanoparticles of chrome citrate. The developers declared that these microfertilizers is safe and environmentally friendly, as their components are carboxylates of trace

elements obtained by erosion-explosive nanotechnology based on nano- and microparticles of these trace elements, their oxides and their hydroxides ranging in size from 1 nm to 15 μm.

The biological effects and toxicological properties of chrome citrate nanoparticles (CCNP) obtained by erosion-explosive nanotechnology according to the Kaplunenko-Kosinov method have not been sufficiently studied concerning plants of different cultures. Since chrome has positive and adverse effects on plant organisms depending on the state of valence and form of existence in complex compounds, it is important to determine the biological impact of chrome citrate nanoparticles. In this work, we conducted a set of studies on the effects of this is newly synthesized substance using the recommended test system and *Allium cep*a as test object (Viana, 2014; Andrusyshina, 2011).

Analysis of recent publications

Nanoparticles attract the special attention of researchers and specialists in various scientific fields due to their new and unique properties, which are different from the properties of particles in the macroscale of similar composition (Viana, 2014; Huynh, 2020; Tripathi, 2017). The passes of substances to nanometer-scale particles caused a change in their structure, physicochemical and biological properties. Such changes are large specific surface area, small size and variety of shapes, increase in chemical potential of the substance, high adsorption activity, high ability to accumulate. Some metal nanoparticles have biocidal, electrical, catalytic and magnetic properties, which is the basis of their classification (Viana, 2014). Studies of the physicochemical properties of a wide range of nanoparticles show that they are the functions of many known and unknown interrelated parameters: technology, time, environment, shape, size and size distribution curve in the nanoscale range, chemical purity, the presence of electrical matter on the surface charge, presence and composition of the stabilizing shell and others (Viana, 2014; Huynh, 2020).

Modern society has already benefited significantly from nanotechnology, as many products with improved properties and a high potential for use have been synthesized and commercialized (Bijali, 2020; Fincheira, 2020). In particular, nanoparticles of different chemical compositions are already used in the detection of toxins and pathogens, diagnosis and treatment of diseases, drug delivery to target organs and cells, product bio labeling, biological and chemical analysis, visualization of results in nonlinear optics, photovoltaics, catalysis, and others. Nanometals are used during the creation of new effective catalysts for the petrochemical industry, for the manufacture of modern sensor systems, in the diagnosis and treatment of infectious and oncological diseases, obtaining materials with bactericidal action, to create new drugs for protection and growth of plants and animals in agriculture and veterinary medicine, in environmental protection (Fakruddin, 2012).

The use of nanopreparation for agriculture is promising (Zhao; 13; Yadav, 2014; Fincheira, 2020), especially of nanoparticles containing essential metals that can be an alternative to mineral fertilizers (Andrusyshina, 2011; Fincheira, 2020). It turned out that some of the applied nanocompounds are important biological regulators of morphogenetic processes in plants. Their role in the processes of growth, differentiation, restoration and regeneration, apoptosis, necrosis, cell survival, and the pathogenesis of chronic inflammatory and degenerative diseases of animals and humans has been proven (Jitao, 2019). It is proved that the lack of trace elements not only leads to lower yields and the development of some diseases in plants and animals but also reduces the quality of food (Arruda, 2015). Studies have shown that some human diseases are associated with a lack of iron, zinc, copper, cobalt, molybdenum, iodine, etc., in food (Jitao, 2019). Therefore, the problem of providing plants and animals with trace elements is of general biological importance.

The active development of nanotechnologies and the introduction of nanoproducts in various sectors of the economy, on the other hand, is of concern to the scientific community about their environmental friendliness and possible toxicity to environmental ecosystems (Andrusyshina, 2011; Yadav, 2014; Fincheira P., 2020). Several studies have shown that some nanoscale particles sometimes exhibit specific toxicity and may be more harmful than substances of the macroscale (Tripathi, 2017). The accumulation of nanoparticles and nanomaterials in the environment can be hazardous because of the potential harm to plants and because they can enter the food chains

(Yadav, 2014). Although the scientific literature on the safety of nanoproducts is growing, the mechanisms by which these materials cause toxicity to natural species, including humans, have not yet been definitively elucidated. There is experimental evidence that nanoparticles such as fullerenes, carbon nanotubes, and metal oxides are toxic to human cells, rodents, and microorganisms (Andrusyshina, 2011; Ivask, 2013). Data on the potential toxicity of nanoparticles to higher plants vary (Yadav, 2014), both positive and negative influences have been reported in the scientific literature (Tripathi, 2017). The interaction between nanoparticles and plants is one of the fundamental problems due to the active introduction of nanoparticles and nanomaterials in the technology of growing crops, long-term storage of agricultural products (Zhao, 2020).

Among biota, plants are suitable for studying the effects of nanoparticles and nanomaterials because they are sensitive to their impact (Tripathi, 2017; Huliaieva H., 2018). It is noted that nanoparticles have several effects on plant physiology and morphology, in particular, on root structure, seed germination, and cellular metabolism (Ivask, 2013; Tripathi, 2017). Some of the nanoparticles inhibit growth, induce oxidative stress, morphogenetic abnormalities and cause clastogenic disorders in some plant species (Huliaieva H., 2018). The size, shape, and surface coating of nanoparticles and nanomaterials have been shown to play an essential role in determining their level of phytotoxicity. Dose, route of administration, type of dispersion medium, and environmental exposure are also essential factors in the formation of phytotoxicity of nanoparticles (Huliaieva H., 2018; Zhao, 2020).

Analysis of work on the phytotoxicity of different nanoparticles and nanomaterials on different plant species shows that the results of their studies are very different (Ivask, 2013; Huliaieva H., 2018; Zhao, 2020). This may be due to the different reactions of the plant depending on their species and the nature and mechanism of action of the studied nanocompound. Before that, most studies to date have focused on the microscopic detection of phytotoxic effects of nanoparticles. Obviously, additional research is needed, including methods and analyzes made using more modern tools (Zhao, 2020).

For a comprehensive study of the toxicity of nanoparticles of various natures, some plant species began to be used as test objects. The mechanisms of toxicity for different types of nanoparticles on plant systems, their absorption mechanisms, distribution in tissues and organs, and subsequent migration on food chains have been studied within this direction (Zhao, 2020). In the works of various authors, recommendations, methods, and methods for assessing the phytotoxicity of nanoparticles by morphoanatomical, physiological, biochemical, and molecular characteristics of plants (Huliaieva, 2018; Ivask, 2013; Yadav T., 2014; Tripathi, 2017). In particular, the phytotoxic effect of Argentum and zinc oxide nanoparticles on *Allium cepa* onion and *Zea mays* corn, *Cucumis sativus cucumber*, and *Lycopersicum esculentum* tomatoes was established by the growth test method. Nanoparticles are also evaluated for their ability to induce cytotoxic and genotoxic effects in the apical meristem of the roots of *A. cepa* bulbs. The mitotic index, the frequency of chromosomal mutations, metaphase disturbances, ruptures, and micronuclei formation were determined in these studies (Kumari M., 2011). Phytotoxicity of dispersed nanoparticles are tested in vitro and in vivo on the seeds of some plants treated with different concentrations, in which root length, germination effect, adsorption, and accumulation of nanoparticles in plant systems were determined. Evaluation of the effect of nanoparticles on the concentration of proteins, DNA, and thiobarbituric acid was performed by biochemical studies, which analyze the functional and conformational changes of biomolecules (Tripathi, 2017). It has been found that some nanoparticles can induce or decrease the regulation of marker genes, affect the transport and retention of water, cell wall formation, and cell division (Tripathi, 2017). Several studies indicate that the phytotoxicity of nanoparticles depends on various factors: their size, shape, carrier, coating, and experimental methods (Yadav T., 2014; Ivask, 2013).

Therefore, nanoparticles, depending on the physicochemical nature, can induce various changes in morpho-anatomical, physiological, biochemical, and molecular characteristics in plants. The literature data suggest that a larger number of engineered nanoparticles, although it has significant advantages in various areas and areas of human activity, which requires factual

information and a science-based database on their toxicity in the use of biosafety and reduction of harmful risks during use and consumption. Thus, the research of the influence of nanoparticles containing important elements for processes of plant morphogenesis, which are planned for use in agricultural cultures production technologies, is a relevant topic, as it is possible to determine the safety and risks of their practical application.

The purpose of the research

The study aims to explore the biological effect of different concentrations of chrome citrate nanoparticles to morphogenesis of the test object *A. cepa*.

Material and methods of the research

The object of study. CCNP were synthesized by erosion-explosive nanotechnology according to the Kaplunenko-Kosinov method (see the patent of Ukraine for a utility model No. 38391) (Kosinov, 2009).

Patent owners note that the drug does not contain other substances, and nanoparticles of essential metal are fully involved in the chemical reaction of synthesis with citric acid salts, resulting in a product of high chemical purity and, most importantly, the resulting compound does not contain free nanoparticles (Kosinov, 2009).

The drug nanoparticles of chrome citrate with a concentration of 1 g/l were used for research. Working CCNP solutions with concentrations of 0.01 to 10 mg/l were prepared by diluting the original preparation with distilled water.

Determination of phytotoxicity for chrome citrate nanopartices was performed by growth *Allium* test using a standard plant test system *A. cepa* (L.) (Fiskesjo, 1997). The phytotoxicity of chrome citrate nanoparticle solutions against *A. cepa* was determined in the concentration range of 0.01 to 10.0 mg/l with a modified variant: the test object *(A. cepa*) bulbs was placed in solutions of nanoparticles of different concentrations without prior root germination (Konotop, 2019).

Laying down the experiment. In control and experimental (at least four analytical replicates and three biological replicates), *A. cepa* bulbs of the Stuttgart variety were placed in 15-ml tubes with working CCNP solutions so that their bottom was utterly immersed. Whole, intact, identical in size (diameter 16 ± 2 mm) and weight (2.90±0.10 g) bulbs of *A. cepa* without green shoots were selected for the experiment.

Exposure of *A. cepa* bulbs in working CCNP solutions was carried out at room temperature (20±2 °C) and dark conditions for five days (to achieve roots of at least 20 mm). Distilled water served as a control.

The overall toxicity of chrome citrate nanoparticle solutions was determined by visually analyzing the turgescence, color, and shape of the germinated roots of *A. cepa* bulbs (on the fifth day of exposure) in control and experimental variants (Konotop, 2019; Lin, 2007).

The phytotoxicity of chrome citrate nanoparticle solutions was determined by growth assay (Fiskesjo, 1993). At the end of the exposure time (after five days), the length (in mm) of the sprouted roots of *A. cepa* bulbs was measured in the control and experimental variants.

The mean value (M) and its error (m) of bulb root length for each experimental (ME) and control (Mk) variants were calculated. The results were recorded in the appropriate table. The Student's test assessed the significance of the difference between the control and experimental variants.

The phytotoxicity index (IFT) of chrome citrate nanoparticle solutions, which is an indicator of the growth activity of the roots of *A. cepa* bulbs, was calculated by the formula:

$$IFT = \frac{Mk - M_E}{Mk} \times 100\%$$

where:

$IFT_{\%}$ – phytotoxicity index of the sample;

M_K – the magnitude of the test reaction in the control sample;

M_E – the value of test reactions in the test sample.

The phytotoxicity of nanoparticles was assessed on a five-point scale (Konotop, 2019) : 0 - 20% – no or low level of toxicity; 20.1-40% - the average level of toxicity; 40.1–60 – above average toxicity; 60.1–80 – high level of toxicity; 80.1-100 – the maximum level of toxicity.

The formula determined the tolerance index (IT %) of the roots of the bulbs of *A. cepa* concerning solutions of nanoparticles determined by the formula (Konotop, 2019):

$$IT = \frac{Md}{Mk} \times 100\%$$

where:

M_d is the average value of the root length of the experimental variant;

M_k is the average value of the root length of the control variant.

Determination of CCNP cytotoxicity by Allium test. The cytotoxicity of a CCNP solution was understood as inhibition of cell proliferation and growth of the test object. Its indicator is the mitotic index (MI) of the cells of the meristem of the roots of the bulbs of *A. cepa* (Fiskesjo, 1997). The mitosis modifying effect of aof CCNP solution was understood as a violation of the passage of cells of the meristem of the test object of the phases of mitosis. Its indicator is the phase indices (IF) of the cells of the meristem of the roots of the bulbs of *A. cepa* (Fiskesjo, 1997).

Scheme of analysis. The tips of the roots of the bulbs of *A. cepa* experimental and control variant length of about 10-15 mm were cut off, fixed, stained, and studied microscopically.

Fixation of roots. The cut roots of A. cepa bulbs were placed in sealed containers with Clark solution (mixture of 96% ethanol and glacial acetic acid, 3v: 1v). After 3-5 hours, the material was washed with 70% ethanol and placed in a container with 70% ethanol for long-term storage.

Staining of the roots of *A. cepa* bulbs was carried out with 2% acetoorsein (2 g of orcein was dissolved by heating to secret boiling in 100 ml of 45% acetic acid and filtered). The roots of A. cepa bulbs were placed in beakers with distilled water. For washing after lowering them to the bottom of the cup, they were transferred to porcelain crucibles with dye. The crucible was covered with a glass plate, heated to a secret boil (glass fogging), and left for 2-24 h to stain the cells. Stained roots of *A. cepa* bulbs were used for the preparation of temporary cytological preparations.

Microscopic analysis. Temporary cyst preparations from the roots of A. cepa bulbs were prepared as follows: the meristem 2-3 mm long was cut off from the painted source with a scalpel, placed on a glass slide in a drop of 2% acetoorsein, covered with a cover glass and light tapping of the handle). Preparations of A. cepa roots were analyzed microscopically (at a magnification of 15x8 and 15x40). Cells at different stages of mitosis were visualized, photographed, and counted using a microscope attachment connected to a computer and PVR-PLUS.

Determination of mitotic and phase indexes. Mitotic index (MI%) is an indicator of cells dividing the total number of all cells. Determination of MI was performed by analyzing at least 500 cells (including interphase) meristems of the roots of *A. cepa* bulbs.

The **mitotic index** (MI%) was determined by the formula:

$$IM = \frac{(P + M + A + T)}{N} \times 100\%$$

where:

(P+M+A+T) – the sum of cells that are at the stage of pro-, meta-, ana- and telophase;

N is the total number of counted cells (including interphase).

The **phase index (IP%)** was determined by the formula:

$$IP = \frac{(FN)}{(P + M = A + T)} \times 100\%$$

where: *IP*% – phase index; *FN* – the number of cells at acertain stage of the mitosis phase; *(P + M + A + T)* – the total number of cells at the stages of pro-, meta-, ana- and telophase.

The **cytotoxicity index (CI%)** was determined by the formula:

$$CI = \frac{IM_E - IM_K}{IM_E} \times 100\ \%$$

where :

IM_E – the average value of the mitotic index of the roots of the experimental variant;

IM_K – the average value of the mitotic index of the roots of control.

Statistical analysis of experimental data. The experiments were performed in at least three biological and four analytical replicates. For each sample, the arithmetic mean (M), standard error of the mean (m), Student's test (t), and reliability (p) were determined. The data were considered reliable at a significance level of $p \leq 0.05$.

Results of the research and their discussion

Research of phytotoxicity of CCNP by growth test.

Phytotoxicity of CCNP (in the range of concentrations 0.01–10.0 mg/l), obtained by erosion-explosive technology, was evaluated by the growth *Allium*-test (Fiskesjo, 1993; Fiskesjo, 1997).

A. cepa bulbs and seeds are widely used in tests for toxicity of test substances of synthetic or natural origin (Blinova, 2013; Lin, 2007) to detect the toxicity substances in the environment (water, soil) (Konotop, 2019; Klepach, 2001). In the *Allium*-test analyzes, the growth and mitotic activity of the roots of *A. cepa* bulbs is analysed (Fiskesjo, 1993). It is a widely recognized bioassay method for assessing the toxicity or non-toxicity of a compound, recommended by the International Commission on Protection against Mutagenic and Carcinogenic Compounds along with other methods (Guide, 1985). This method is convenient, easy to perform and gives a more objective assessment of the toxicity for test substances compared to chemical ones, especially in the case presence of upper limits of maximum permissible concentrations of substances (Guide, 1985).

In the first stage of the *Allium*-test, we observed and evaluated the growth activity of the roots of *A. cepa* bulbs during the exposure time (see *Figure 2*). In the fifth day of exposure, we conducted visual analysis of the roots of *A. cepa* bulbs (provided they reach at least 20 mm) turgescence, color, shape (swelling and bending), and their length was measured. The results of observations and measurements are shown in *Tables 1* and *2*. As can be seen from *table 1* and *table 2*, growth activity and morphological parameters of the roots of *A. cepa* bulbs grown in CCNP solutions in the concentration range from 0.01 to 0.10 mg/l, without visible morphological and structural changes (the shape of the roots is elongated and regular, they are light, the tips are slightly pigmented). However, the growth activity of the *A. cepa* bulbs grown in CCNP solutions in the range of concentrations from 0.05 to 1.00 mg/l is reduced. In addition, there is a change in the shape of their roots: they acquire a somewhat tortuous form.

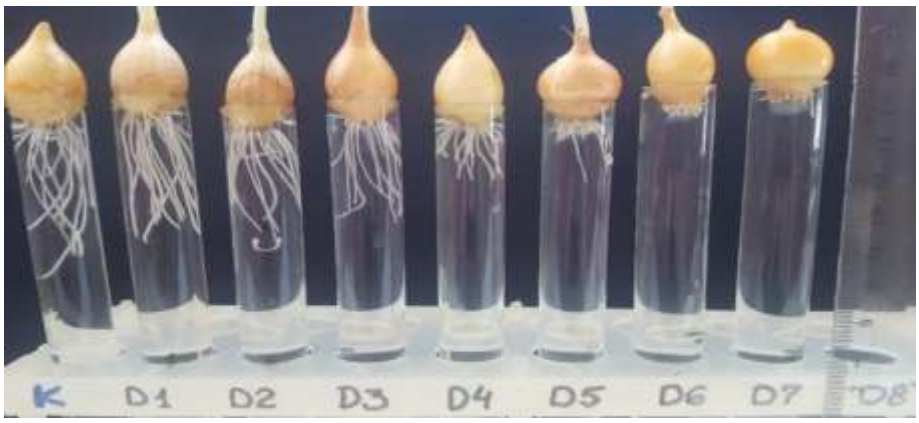

Fig. 1. Type of roots of Allium cepa bulbs on the 5th day of growth on aqueous CCNP solutions: K – control (distilled water); D1 – 0.01 mg/l; D2 – 0.05 mg/l; D3 – 0.10 mg/l; D4 – 0.50 mg/l; D5 – 1.0 mg/l; D6 – 5.0 mg/l; D7 – 10.0 mg/l

Phytotoxicity indexes ($IFT_{\%}$) served to measure the phytotoxic effect of CCNP of different concentrations on the test object. $IFT_{\%}$ is an essential indicator in the initial assessment of the impact of the test substance on the phytoobject, especially in environmental monitoring (Lin, 2007). Thus, CCNP solutions in the concentration range of 0.01–0.05 mg/l do not cause changes in turgescence, color, and shape of the tips of the roots of the bulbs of the test object (see Table 1).

Table 1

Morphological parameters of the roots of *Allium cepa* bulbs grown on aqua solutions of chrome citrate nanoparticles

<table>
<tr><th>Sample</th><th>Concentration of chrome citrate nanoparticles, mg/l</th><th>Turgescence</th><th>Shape of the roots</th><th>Color of the roots</th></tr>
<tr><td>K</td><td>Control</td><td rowspan="4">Normal</td><td rowspan="4">Elongated correct</td><td rowspan="4">Bright with light tips</td></tr>
<tr><td>D1</td><td>0.01</td></tr>
<tr><td>D2</td><td>0.05</td></tr>
<tr><td>D3</td><td>0.10</td></tr>
<tr><td>D4</td><td>0.50</td><td>Normal</td><td>Elongated, slightly tortuous</td><td>Bright with light tips</td></tr>
<tr><td>D5</td><td>1.0</td><td>Normal</td><td>meandering</td><td>Bright with light tips</td></tr>
<tr><td>D6</td><td>5.0</td><td colspan="3" rowspan="2">There is no growth</td></tr>
<tr><td>D7</td><td>10.0</td></tr>
</table>

The growth activity of its roots does not differ significantly compared to the control and experimental variants (see Table 2). These morphometric parameters of the test object indicate that at the body level of phytotoxicity of CCNP in the range of 0.01-0.05 mg/l is not observed. The levels of tolerance (IT%) of the roots of A. cepa bulbs to these concentrations of nanoparticles is high and is 80.84% and 92.81%, respectively.

As can be seen from *Tables* 1 and 2 and *Figures* 2, higher concentrations of CCNP (in the range of 0.1 to 5.0 mg/l) have higher levels of phytotoxicity (see *Figure 2*) relative to the growth activity of the roots of *A. cepa* bulbs. In particular, it was determined that CCNP in the concentration range from 0.05 to 0.10 mg/l have a phytotoxic effect of medium level, in the concentration range from 0.10 to 0.50 mg/l – above-average level, from 0, 50 to 1.00 mg/l – high level concerning *A. cepa.* In the range of nanoparticle concentrations 5–10 mg/l – the growth of the roots of *A. cepa* bulbs is almost not observed: on a dark background instead of roots, thin short root hairs are visible, which easily fall off when touched from the bottom of the bulb. Thus, concentrations of CCNP of 5 mg/l and above cause phytotoxicity of the maximum (≈100) level relative to the growth activity of the test object *A. cepa.*

As we can see (see *Table 2, Figure 2*), the tolerance of *A. cepa* bulb roots to higher concentrations of CCNP decreases accordingly. Both values – phytotoxicity of higher concentrations of nanoparticles and phytotolerance of roots of *A. cepa* bulbs are negatively correlated with each other (R = -1) (see *Figure 2, B*).

Therefore, CCNP solutions in the concentration of 0.01 to 0.05 mg/l do not cause phytotoxicity in the studied plant object A. cepa. Phytotolerance to these concentrations of nanoparticles in A. cepa is high and reaches 80.84% – 92.81%. The obtained indicators of

phytotolerance of the studied object A. cepa to CCNP indicate the prospects of their use in the range of concentrations of 0.01 – 0.05 mg/l in the technologies of growing agricultural plants.

Table 2

Morphometric parameters of the roots of *Allium cepa* bulbs grown on solutions of chrome citrate nanoparticles

Sample	Concentration of nanoparticles, mg/l	Length of roots, mm M ± m	* IFT%, (phytotoxicity level)	*IT%
K	Control	33,4±3,0	0	100
D1	0.01	31.0±3.2 t=0.55; p=0.58	7.19 (missing)	92.81
D2	0.05	27.0±3.1 t=1.48; p≤0.14	19.16 (missing)	80.84
D3	0.10	24.5±2.4 t=2.33; p=0.02	26.65 (average)	73.35
D4	0.50	18.2±2.1 t=4.15; p≤0.01	45.51 (above average)	54.49
D5	1.00	11.30±1.9 t=6.22; p≤0.01	66.17 (high)	33.83
D6	5.0	0.00	100 (maximum)	0
D7	10.0			

IFT% - phytotoxicity index
IT% - level of tolerance

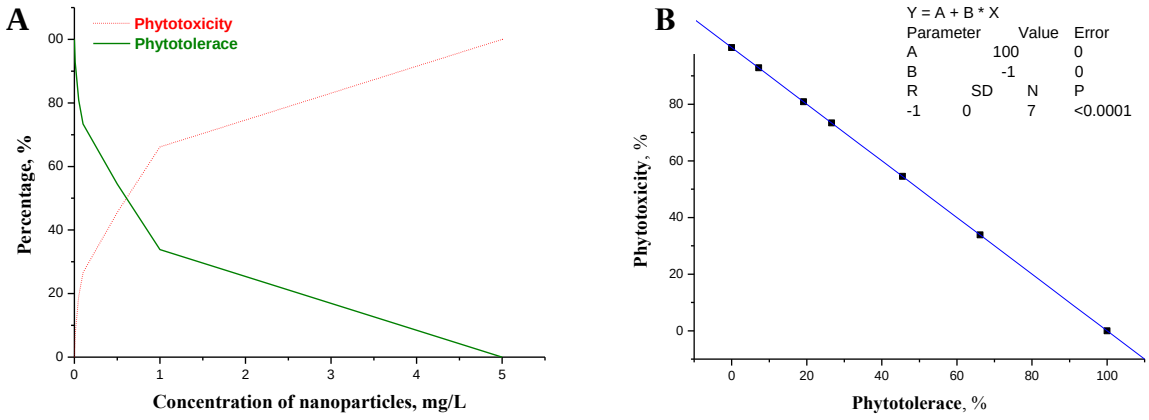

Fig. 2. A. Phytotoxic effect of CCNP on the growth activity of the roots of A. cepa bulbs B. Correlation between phytotoxicity of increasing concentrations (from 0 to 5.0 mg/l) of CCNP and phytotolerance of A. cepa bulb roots to them

Research of cytotoxicity of CCNP by *Allium* test.

One of the indicators assessing the degree of influence of various factors on changes in the structural and functional characteristics of cells is the level of mitotic activity (Fiskesjo, 1997). In this regard, we determined the mitotic activity in the proliferative cells of the apical meristem of *A.*

cepa roots. The cytotoxicity of CCNP solutions was assessed in the *Allium* test by the mitotic activity of the meristem cells of the root bulbs of the test object. At the same time, in the *Allium* test we analyzed the mitosis modifying effect of nanoparticle solutions, which meant as a violation of the passage of cells of the meristem of the test object of the phases of mitosis, and the cytotoxic effect – inhibition of cell proliferation.

The mitotic index (MI%) of the apical meristem of the test object was the indicator of mitotic activity, and the cytotoxicity index was the indicator of cytotoxicity for nanoparticle solutions relative to the test object.

For this purpose, cytopreparations were prepared from apical parts (meristems) of *A. cepa* bulb roots grown in CCNP solutions with concentrations that did not cause high phytotoxicity to the test object in the growth test. Because the cytotoxicity of the test substances can be manifested at different stages of the cell cycle, the cell distribution of the primary meristem of *A. cepa* depending on the phase of mitosis was studied (Fiskesjo, 1997). To detect targets of mitosomodifying action of solutions nanoparticles, the phase indices of the cells of the meristems of the test object at different stages of mitosis were determined (see *Figure 3* & *Figure 4*).

As can be seen (*Figure 3*), the mitotic indices of the test object meristems of experimental samples D1 and D2 are close to the control (MI=80.60), and the cytotoxicity indices of the corresponding CCNP solutions are low (1.71 and 3.83, respectively). The phase indices of pro-, meta-, ana- and telophase meristems of the test object in experimental samples D1 and D2 are also close to control and fluctuate within the norm. Thus, the mitotic and phase indices of mitotic activity of *A. cepa* meristem indicate the absence of cytotoxic and mitosis modifying effect of CCNP in the concentration range of 0.01 - 0.05 mg/l relative to the test object.

The mitotic index of the meristem for the test object D3 (see Figure 3) decreases to 62.88, and the index of cytotoxicity, respectively, increases to 21.98. As can be seen from Figure 4, the indices of the phase indices of the meristem of A. cepa of this experimental sample D3 are also different from the control. Mitotic cells have the highest rate in the prophase state (54.13%), indicating a delay in the processes at this stage of mitosis. The prophase index of variant D3 increases by 7% compared to the control (PIK = 47.00%) and significantly exceeds it by 1.15 times. The phase indices of meta-, ana- and telophases, respectively, decrease compared to the control by 2-3%. Lower phase indices indicate a delay in the mitotic activity of the meristems of the test object at the prophase stage, which may be associated with the negative effect of 0.10 mg/l CCNP solution on the processes of chromosome condensation and redistribution (Konotop, 2019).

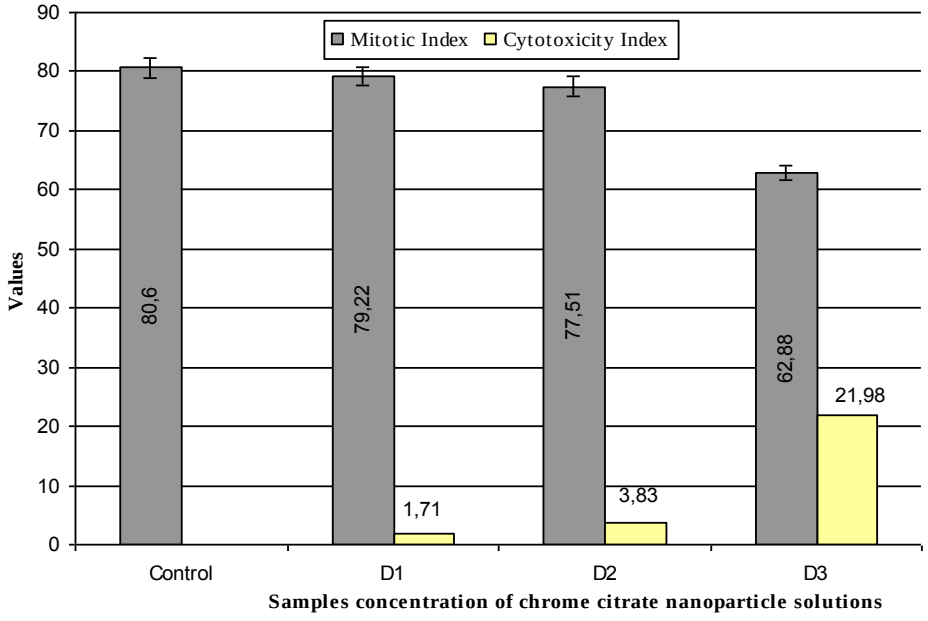

Fig. 3. Mitotic and cytotoxicity indexes of the apical meristem of the roots of Allium cepa bulbs grown on solutions of chrome citrate nanoparticles:
D1 – 0.01 mg/l; D2 – 0.05 mg/l; D2 – 0.1 mg/l

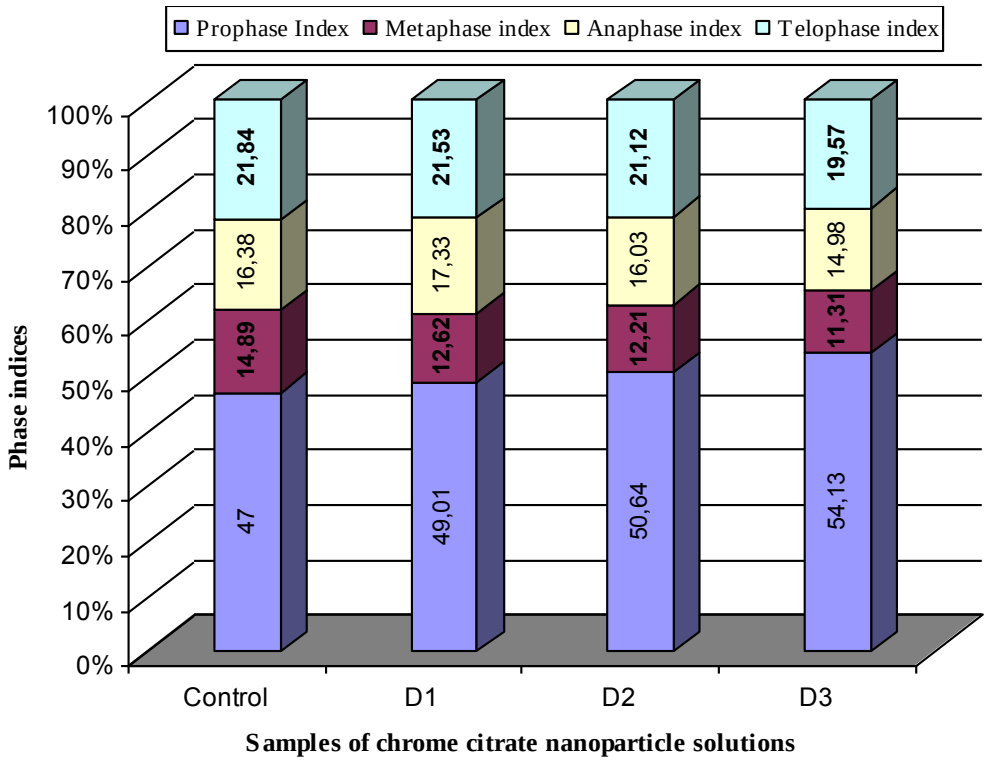

Fig. 4. Mitotic activity of cells in the apical meristem of the roots of Allium cepa bulbs grown on solutions of chrome citrate nanoparticles: D1 – 0.01 mg/l; D2 – 0.05 mg/l; D2 – 0.1 mg/l

Thus, the results of the studies indicate an average level of cytotoxicity of 0.10 mg/l CCNP solution relative to the test object, which is consistent with our previous data (see above): the level of phytotoxicity of the same solution of nanoparticles close to the growth activity of roots *A. cepa* is average.

Conclusions

Growth *Allium* test revealed that CCNP solutions in the concentration range of 0.01-0.05 mg/l did not cause phytotoxic effects on *A. cepa* at the organism level: they do not cause changes in the turbidity, color, and shape of the tips of their roots, growth activity – the length of the roots of the test samples does not differ significantly from the control. The phytotolerance of the roots of *A. cepa* bulbs to these concentrations of nanoparticles is high and reaches 80.84% – 92.81%.

It was determined that CCNP in the range of concentrations of 0.01-0.05 mg/l in the *Allium* test did not show mito- and cytotoxicity and mitosis modifying effect on the proliferative activity of cells of the test object meristem: mitotic and phase indices are within normal limits; cytotoxicity indices are within the background values.

It was found that CCNP in the concentration range of 0.05-10.00 mg/l caused phytotoxic effects of different levels on the growth activity of the test object roots. In particular, the phytotoxicity of nanoparticles in the concentration range from 0.05 to 0.10 mg/l is of average level; in the field of concentrations from 0.10 to 0.50 mg/l – above average, in the range of concentrations from 0.50 to 1.00 mg/l – high; in the field of concentrations from 5 to 10 mg/l maximum.

It was determined that CCNP at a concentration of 0.1 mg/l showed cytotoxicity and mitosis modifying effect on the test object: the mitotic index of the meristem *A. cepa* decreases, phase indices differ from standard, there is a delay in chromosome spiraling at the level prophase: the level of cytotoxicity of this solution of nanoparticles against *A. cepa* is average.

The obtained research results confirm the possibility of using CCNP in technologies of growing crops in the range of biologically acceptable concentrations (0.01-0.05 mg/l), which do not reveal any manifestations of phyto-, mito- and cytotoxicity.

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Chapter 3. ACTUAL PROBLEMS AND EDUCATIONAL ASPEMTS OF THE SPEMIALTY 226 "PHARMACY, INDUSTRIAL PHARMACY"

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Abstract. *Ensuring the monitoring of the quality of educational services and the educational process in higher education institutions (HEIs), that offer Educational Programs to train specialists in medical and pharmaceutical branches is one of the health care obligations that Ukraine as a state undertakes. Traditionally, specialists for pharmaceutical manufacturing were trained mostly at the HEIs, which were subordinated to the Ministry of Education and Science of Ukraine (MES), and those for pharmacies and hospital pharmacies were trained at the HEIs, subordinated to the Ministry of Health of Ukraine (MOH).*

In 2016, a single specialty 226 "Pharmacy" was introduced, and in 2017 the title name of the specialty was specified as 226 "Pharmacy, Industrial Pharmacy". The draft of Resolution of the Cabinet of Ministers of Ukraine (CMU) "On the Amendments to the List of a branch of knowledge and specialties for higher education" provides dividing of the specialty 226 "Pharmacy, Industrial Pharmacy" into two separate specialties: 226 "Pharmacy", which remains in the branch of knowledge 22 "Health Care", and 188 "Industrial Pharmacy", which potentially will be transferred to the branch of knowledge 18 "Manufacturing and Technology". The dividing of specialty into two separate ones is pertinent; however, the problem of transfer significantly affects the training of highly qualified human resources. That is why it is important to take out this problem from a limited circle of specialists in the pharmaceutical branch to the general public.

The research aimed to study and summarize the legislation on the current state of specialists training for the pharmaceutical branch by the specialty 226 "Pharmacy, Industrial Pharmacy", as well as the search for possible ways to solve the educational problem. The following methods were applied: meta-analysis, desk research, comparative analysis and modeling.

It is established the necessity to save the specialty "Industrial Pharmacy" in the field of knowledge 22 "Health Care", as its removal levels the results of specialists training and makes it impossible for the functioning pharmaceutical industry of Ukraine. As a result of this process, obstacles will be created for the research realization, development, and implementation of new medicines and generic drugs. It is extremely important to save the specialty "Industrial Pharmacy" in the field of knowledge 22 "Health Care". This is the only possible way to guarantee the quality of the drugs according to the Complex of Good Pharmaceutical Practices (GMP, GLP, GCP, GDP, GPP, GSP, GEP, GPEP) at all stages of its promotion from manufacturer to the patient, that will facilitate scientific research and development of new safe, effective, high-quality medicines, and also will allow providing training of highly skilled specialists for pharmaceutical branch.

Keywords: *healthcare, pharmacy, industrial pharmacy, legislative documents, branch of knowledge, specialty*.

Introduction. Maintaining the health of the citizens of their country is one of the main tasks of the state. It should be noted that our state, according to the Constitution of Ukraine and other regulations, undertakes a number of very important obligations regarding health care (Constitution of Ukraine, 2020; Law of Ukraine No. 27, 2021; Law of Ukraine No. 23, 2019). Among them are not only functions related to organizational, legislative, validation, certification, licensing, controlling processes, but also to ensure monitoring the quality of educational services and the educational process in higher education institutions (HEIs).

It is known, the most of the HEIs are subordinated to the Ministry of Education and Science of Ukraine (MES) and only medical and pharmaceutical HEIs are subordinated to the Ministry of Health of Ukraine (MOH). However, the adoption of a number of legislative acts for the past five years has led to certain problems in the pharmaceutical industry, which begin from the specialist's training and the provision of quality educational services. Traditionally, specialists for pharmaceutical manufacturing were trained mostly at the HEIs, which were subordinated to the MES, and those for pharmacies and hospital pharmacies were trained at the HEIs, subordinated to the MOH. But the situation has changed dramatically since 2015/2016.

Analysis of recent publications. According to the Resolution of the Cabinet of Ministers of Ukraine (CMU) "On approval of the list of the branch of knowledge and specialties for which the training of higher education applicants" on 29.04.2015, specialty "Pharmaceutical Technology" was removed, renamed and referred to another branch of knowledge – 22 "Health Care", specialty 226 "Pharmacy" (Resolution of Cabinet of Ministers No. 266, 2015). In 2017, according to the Resolution of the CMU No. 53 on 01.02.2017, this specialty was renamed as 226 "Pharmacy, industrial pharmacy" (.Resolution of Cabinet of Ministers No. 53, 2017). The problematic issues of pharmaceutical education in Ukraine are well known to both scientists and practitioners in the field. The most comprehensive issue of the quality training of specialists nowadays was set out in the speech of the Rector of the National University of Pharmacy (NUPh), Professor Alla Kotvitska (Kotvitska, A. A., 2021).

However, the understanding of artificially created problems, unfortunately, does not allow to easy and quick their solving, because the decisions, approved by legislation, are related to the interests of various structures, and also have a certain period of validity; they are difficult to change immediately.

The purpose of the research. The research aimed to study and summarize the legislation on the current state of specialists training for the pharmaceutical industry by the specialty 226 "Pharmacy, Industrial Pharmacy", as well as the search for possible ways to solve the educational problem. Study objects included legislative acts on training of specialists by the specialty 226 "Pharmacy, Industrial Pharmacy"; study and analysis of educational programs of the HEIs that offered such training. The following methods were applied: meta-analysis, desk research, comparative analysis and modeling.

Presentation of the main material As a result of the renaming and transferring of the specialty, in which the training of technological engineers for pharmaceutical companies was provided, this direction of specialist's training actually found itself in the field of knowledge 22 "Health Care" in framework specialty 226 "Pharmacy".

However, in 2017, when the name of the specialty 226 "Pharmacy, Industrial Pharmacy" was implemented, a conflict of interest arose. Such a title name of the specialty theoretically foresaw that the graduated specialist will be able to immediately work in both pharmacies and industrial pharmaceutical companies. The problem was that no university could provide such education. In order to train a specialist of this level, it would be necessary at first organize learning in medical (pharmaceutical) HEIs, subordinated to the MOH, and then, to obtain the qualification of technological engineer, – in HEIs, subordinated to the MES.

Actually, all professionals in the field, scientists, government agencies understand the requirement for dividing the specialty 226 "Pharmacy, Industrial Pharmacy" into two separate specialty "Pharmacy" and "Industrial Pharmacy". However, this is not easy to implement for technical reasons. All digital codes are fully used in field of knowledge 22 "Health Care" (Table 1).

On August 13, 2021, the MEH proposed for public discussion a draft of Resolution "On the Amendments to the List of a branch of knowledge and specialties for higher education", provides dividing of the specialty 226 "Pharmacy, Industrial Pharmacy" into two separate specialties: 226 "Pharmacy", which remains in the branch of knowledge 22 "Health Care", and 188 "Industrial Pharmacy", which potentially will be transferred to the branch of knowledge 18 "Manufacturing and Technology".

Table 1

The list of fields of knowledge and specialties on which training of high education applicants is carried out

Code and name of the field of knowledge	№	Title name of specialty in Ukraine		According to the International Standard Classification of Education	
		Code	Title name of specialty	Code	Title name of the corresponding detailed branch
22 Health Care	1	221	Dentistry	0911	Dental Studies
	2	222	Medicine	0912	Medicine
	3	223	Nursing	0913	Nursing and Midwifery
	4	224	Medical Diagnostic and Treatment Technology	0914	Medical Diagnostic and Treatment Technology
	5	225	Medical Psychology	0313	Psychology
	6	226	Pharmacy, Industrial Pharmacy	0588	Inter-disciplinary programs and qualifications involving natural sciences, mathematics and statistics
				0711	Chemical Engineering and Processes
				0916	Pharmacy
	7	227	Physical Therapy, Ergotherapy	0915	Therapy and Rehabilitation
	8	228	Pediatry	0912	Medicine
	9	229	Public Health	0413	Management and Administration

The division of specialty 226 "Pharmacy, Industrial Pharmacy" into two separate ones, is expedient and timely. However, in our strong belief, the specialty "Industrial Pharmacy" should be saved in the field of knowledge 22 "Health Care". For this reason we propose to enter a separate code 226.1 for specialty "Industrial Pharmacy". At the same time, it should be predicted that the specialist's training in the specialty "Industrial Pharmacy" can be provided in the HEIs, which are subordinated to both the MOH and the MES. The training of the last one should be conducted according to the two-degree system of education (the first – bachelor's degree and the second – master's degree of higher education), and, respectively, the defense of bachelor's / master's qualification project should remain as the form of final certification. It would be expedient legislatively to provide an opportunity for applicants for higher education in the direction "Pharmacy" and "Industrial Pharmacy" to receive a second higher pharmaceutical education across passing the STEP 1 exams in the 4th year of study for direction "Industrial Pharmacy" and across defense the bachelor's qualification project for direction "Pharmacy" and the possibility of parallel training in the appropriate specialization.

Another alternative way may be a proposal to release the code 228 for separated specialty "Industrial Pharmacy" by combining the specialties "Medicine" and "Pediatrics" into a single specialty – "Medicine", as according to the International Standard Classification of Education, code and title name of the relevant detailed field is the same – 0912 Medicine.

It is extremely important to save the specialty "Industrial Pharmacy" in the field of knowledge 22 "Health Care". This is based on a deep understanding of a single logically related legal framework, which is formed for both the pharmaceutical industry and the intermediary level (pharmaceutical companies and pharmacies) in the health care field in Ukraine.

Relevant specialist's training within the given specialty of the second (master's) degree of higher education based on the first (bachelor's) degree is provided at the leading domestic universities, including: Kyiv National University of Technologies and Design (Kyiv), Lviv Polytechnic National University (Lviv), Institute of Chemicals Technologies of the Volodymyr Dahl East Ukrainian National University (Dnipro), Ukrainian State University of Chemical Technology (Kyiv), Odessa Polytechnic State University (Odessa), which are subordinated to the MES, as well as the NUPh (Kharkiv), which is subordinated to the MOH.

According to the resolution of the CMU № 497 on 19.05.2021, specialty 226 "Pharmacy, Industrial Pharmacy" of the branch of knowledge 22 "Health Care" included in the List of specialties for which an single state qualifying examination (SSQE) is a form of final certification of master's degree applicants (Resolution of Cabinet of Ministers No. 497, 2021). According to the resolution of the CMU No. 334 on 28.03.2018, the MOH conducts an SSQE in the STEP 2 format for master's degree applicants, who are training based on the complete general secondary education (Resolution of Cabinet of Ministers, 2018).

The training specialists of the second (masters) degree of higher education in the field of "Industrial Pharmacy" is provided based on the bachelor's degree program, which does not require passing the STEP 1 exam. Nowadays, for such a master's degree program the SSQE STEP 1 and SSQE STEP 2 have not been developed. At the same time, it is determined that the defense of the master's qualification project is the only a form of certification of master's degree applicants. The aim of the program is to acquire the general and special competencies for professional activities in the relevant position in the field of industrial production of medicines. From 2022 the SSQE becomes a mandatory component of the individual curriculum of the student, and the curricula for the 2021-2022 academic years at the Lviv Polytechnic National University have already been approved, that's why making appropriate changes is impossible. It is, therefore, necessary to provide for an intermediate period for the implementation of the STEP 1 and STEP 2 exam systems.

For making comparative analysis, we considered the list of disciplines approved by the Educational and Professional Program (EPP) of Danylo Halytsky Lviv National Medical University, for the training of specialists of the second (master’s) degree of higher education of the specialty 226 "Pharmacy, Industrial Pharmacy", and a list of educational components for the applicants of first (bachelor's) and for the second (master's) degree of higher education of the specialty 226 "Pharmacy, Industrial Pharmacy", trained at the Lviv Polytechnic National University (Tables 2, 3, 4, and 5).

Table 2

The list of educational components of EPP "Pharmacy, Industrial Pharmacy" in Danylo Halytsky Lviv National Medical University

Subject matter code	Curriculum unit (subject matter, course projects, practical training, qualification project)	Number of credits	Form of final control
1	2	3	4
Obligatory EPP component			
Humanitarian training			
OC 1.	The Ukrainian Language (professional-oriented)	3	Credit
OC 2.	History of Ukraine and Ukrainian Culture	3	Credit
OC 3.0.	Philosophy	3	Credit with the mark
OC 3.1.	Bioethics	0,5	Credit
OC 4.	The Foreign Language (professional-oriented)	5	Examination
	Total	***14,5***	
Fundamental training			
OC 5.	Latin	3	Credit
OC 6.	Human Anatomy	3	Credit
OC 7.	Physiology	4	Examination
OC 8.	Biological physics and physical methods of analysis	4,5	Examination
OC 9.	Biology with essential genetics	3	Examination
OC 10.	Higher mathematics and statistics	4	Examination
OC 11.	General and inorganic chemistry	9	Examination
OC 12.	Information technologies in pharmacy	4	Credit with the mark
OC 13.	Analytical chemistry	8	Examination
OC 14.	Organic chemistry	8	Examination
OC 15.	Pharmaceutical botany	5	Examination

OC 16.	Microbiology and essential immunology	5	Examination
OC 17.	Physical and Colloid chemistry	5	Examination
OC 18.	Biological chemistry	6	Examination
OC 19.	Ethics an deontology in pharmacy	2	Credit
OC 20.	Pathological physiology	5	Examination
OC 21.	Computer simulation in pharmacy	3	Credit
OC 22.	Medicine of extreme conditions	2	Credit
	Total	***83,5***	
	Vocational training		
OC 23.	Hygiene in pharmacy and ecology	3	Credit
OC 24.	Pharmacology	9	Examination
OC 25.	Pharmacognosy	9	Examination
OC 26.	Law and legislation in Pharmacy	2	Credit
OC 27.	Pharmaceutical chemistry	14	Examination
OC 28.	Pharmacotherapy with pharmacokinetics	3	Examination
OC 29.	Health care management	3	Credit
OC 30.	Technology of medicines	13	Examination
OC 31.	Organization and economics in pharmacy	7	Examination
OC 32.	The study of pharmaceutical and medical commodities	4	Examination
OC 33.	Management and marketing in pharmacy	9	Examination
OC 34.	Clinical pharmacy and pharmaceutical care	9	Examination
OC 35.	Toxicological and forensic chemistry	6	Examination
OC 36.	Biopharmacy	3	Credit
OC 37.	Appropriate practices in pharmacy	3	Credit
OC 38.	The resource study of medicinal plants	3	Credit
OC 39.	Standardization of medicines	3	Credit
OC 40.	Technology of cosmetics	3	Credit
OC 41.	Pharmaceutical biotechnology	3	Credit
OC 42.	Quality assessment system in pharmacy	3	Credit
OC 43.	Social pharmacy	3	Credit
OC 44.	Military oriented pharmacy training	3	Credit
	Total	*118*	
	Industrial practice		
OC 45.	Introductory practice on the organization and economics in pharmacy	1,5	Credit with the mark
OC 46.	Propaedeutic practice on pharmacy medicines technology	1,5	Credit with the mark
OC 47.	First preliminary aid with the introductory medical practice	3	Credit
OC 48.	Field practice in pharmaceutical botany	3	Credit with the mark
	Total	*9*	
	Total for the vocational training	***127***	
General amount of the obligatory program units		**225**	
Elective components of the Curriculum*			
Elective module 1			
EM 1.1.	The Foreign Language (second)	9	Credit
EM 1.2.	The English Language	12	Credit
EM 1.3.	Modern problems of molecular biology	3	Credit
EM 1.4.	Modern problems of biophysics	3	Credit
EM 1.5.	Physical and chemical methods of analysis	3	Credit
EM 1.6.	Biological role of vital elements	3	Credit
EM 1.7.	Theory of knowledge and pharmacy	3	Credit
EM 1.8.	Sociology and medical sociology	3	Credit
EM 1.9.	Psychology of communication	3	Credit
EM 1.10.	Theory and practice of professional communication	3	Credit

EM 1.11.	History of medicine and pharmacy	3	Credit
EM 1.12	Security of vital activity and biological security	3	Credit
EM 1.13.	Political science	3	Credit
EM 1.14.	Fundamentals of Cristian ethics and moral	3	Credit
EM 1.15.	Basics of computer technologies in pharmacy	3	Credit
EM 1.16.	Pharmaceutical informatics and statistics	3	Credit
EM 1.17.	Principles of systemic analysis	3	Credit
EM 1.18.	Basics of chemical metrology	3	Credit
EM 1.19.	Identification of organic compounds	3	Credit
EM 1.20.	Modern analytical laboratory practice	3	Credit
EM 1.21.	Principles of social psychology	3	Credit
EM 1.22.	Medicinal plants of pharmacy	3	Credit
EM 1.23.	First aid in the pharmaceutical institutions	3	Credit
EM 1.24.	Pathological physiology of civilization diseases	3	Credit
EM 1.25.	Chemistry of medicines	3	Credit
EM 1.26.	Modern methods of investigating biological systems	3	Credit
EM 1.27.	Metabolism of drugs	3	Credit
EM 1.28.	World pharmaceutical industry	3	Credit
EM 1.29.	Mechanism of pharmacological activity and toxicity of drugs	3	Credit
EM 1.30.	Pharmaceutical aspects of toxicomania	3	Credit
EM 1.31.	Pharmaceutical aspects of phytotherapy	3	Credit
EM 1.32.	Microorganism in biotechnological processes	3	Credit
EM 1.33.	Basics of economics in pharmacy	3	Credit
EM 1.34.	Ethical problems in pharmacy	3	Credit
EM 1.35.	Limited batch production of medicine	3	Credit
EM 1.36.	Drugs development	3	Credit
EM 1.37.	Theoretical principles of synthesis and the relation between the structure and the effect of medicines	3	Credit
EM 1.38.	Quality control of medicines	3	Credit
EM 1.39	Investigation of the pharmaceutical market	3	Credit
EM 1.40.	Side effects of drugs	3	Credit
EM 1.41.	Pharmacogenetics	3	Credit
EM 1.42.	Pharomacology	3	Credit
EM 1.43.	Basics of clinical medicine	3	Credit
EM 1.44.	Basics of medical standardization and evidence medicine	3	Credit
EM 1.45.	Drug-related problems	3	Credit
EM 1.46.	Modern medical products	3	Credit
EM 1.47.	Logistic in pharmacy	3	Credit
EM 1.48.	Pharmaceutical aspects of nutriciology (biological active additives)	3	Credit
EM 1.49.	Ecotoxicology	3	Credit
EM 1.50.	Chemical and toxicological analysis	3	Credit
EM 1.51.	Medicinal plants and phytotherapy	3	Credit
EM 1.52.	Physical training	6	Credit
EM 1.53.	Patentology	3	Credit
Elective module 2			
EM 2.1.	Industrial practice in pharmacognosy	3	Credit with the mark
EM 2.2	Industrial practice in the technology of drugs	6	Credit with the mark
EM 2.3.	Industrial practice in organization and economics in pharmacy	5	Credit with the mark
EM 2.4.	Industrial practice in management and marketing in pharmacy	4	Credit with the mark

EM 2.5.	Industrial practice in pharmaceutical chemistry	5	Credit with the mark
EM 2.6	Industrial practice in clinical pharmacy	1	Credit with the mark
EM 2.7.	Industrial practice in clinical pharmacy and pharmaceutical care	5	Credit with the mark
EM 2.8.	Industrial practice in pharmacoeconomics	1	Credit with the mark
EM 2.9.	Industrial practice in pharmaceutical information	4	Credit with the mark
Elective module 3			
EM 3.1.	Methodology of scientific investigations on the technology of drugs based on the subject of Master's degree paper	15	Defense of a Master's Degree Paper
EM 3.2.	Methodology of scientific investigations on pharmaceutical chemistry based on the subject of Master's degree paper	15	Defense of a Master's Degree Paper
EM 3.3.	Methodology of scientific investigations on the pharmacognosy based on the subject of Master's degree paper	15	Defense of a Master's Degree Paper
EM 3.4.	Methodology of scientific investigations on organization and economics in pharmacy based on the subject of Master's degree paper	15	Defense of a Master's Degree Paper
EM 3.5.	Methodology of scientific investigations on the clinical pharmacy based on the subject of Master's degree paper	15	Defense of a Master's Degree Paper
EM 3.6.	Methodology of scientific investigations on the toxicological and forensic chemistry based on the subject of Master's degree paper	15	Defense of a Master's Degree Paper
General amount of the elective program units		**75**	
GENERAL AMOUNT OF THE SYTLLABUS		**300**	

Table 3

The list of educational components of EPP "Pharmacy, Industrial Pharmacy" for the first (bachelor's) degree of higher education in Lviv Polytechnic National University

Subject matter code	Curriculum unit (subject matter, course projects, practical training, qualification project)	Number of credits	Form of final control
1	2	3	4
Common components of educational and professional programs			
1. The cycle of general training			
OC 1.1.	Higher mathematics, part 1	6	Examination
OC 1.2.	The Foreign Language (professional-oriented), part 1	3	Credit with the mark
OC 1.3.	History of statehood and culture of Ukraine	3	Examination
OC 1.4.	Chemistry 1 (general and inorganic chemistry)	5	Examination
OC 1.5.	Chemistry 2 (organic chemistry), part 1	9	Examination
OC 1.6.	Higher mathematics, part 2	6	Examination
OC 1.7.	The Foreign Language (professional-oriented), part 2	3	Credit with the mark
OC 1.8.	The Ukrainian Language (professional-oriented)	3	Examination
OC 1.9.	Physics	7	Examination
OC 1.10.	Physical and Colloid chemistry	7	Examination
OC 1.11.	Chemical methods of analysis of substances	4	Credit with the mark
OC 1.12.	Biology and physiology with basic of anatomy	7	Examination
OC 1.13.	The Foreign Language (professional-oriented), part 3	3	Examination
OC 1.14.	Microbiology	7	Examination
OC 1.15.	Physical and chemical methods of analysis of substances	4	Credit with the mark
OC 1.16.	Biological chemistry and molecular biology	8	Examination
OC 1.17.	Informatics	3	Credit with the mark

OC 1.18.	Philosophy	3	Examination
Total for the cycle		***91***	
2. The cycle of professional training			
OC 2.1.	Introduction to the profession and the basics of professional hygiene	4	Credit with the mark
OC 2.2.	Latin	3	Credit with the mark
OC 2.3.	Methods of organic synthesis	6	Examination
OC 2.4.	Medical botany	6	Examination
OC 2.5.	Toxicological chemistry	4	Examination
OC 2.6.	Drugs technology in pharmacy	6	Examination
OC 2.7.	Engineering and computer graphics	4	Credit with the mark
OC 2.8.	Processes and devices of pharmaceutical manufacturing	5	Examination
OC 2.9.	Fundamentals of labor protection and life safety	3	Examination
OC 2.10.	Pharmacognosy	5	Examination
OC 2.11.	Pharmaceutical chemistry	7	Examination
OC 2.12.	Organization and economics in pharmacy	3	Examination
OC 2.13.	Basic of pharmacology	4	Examination
OC 2.14.	Drugs technology in pharmacy, Course Project	2	Credit with the mark
OC 2.15.	Pharmacognosy, Course Project	2	Credit with the mark
OC 2.16.	Organization and economics in pharmacy, Course Project	2	Credit with the mark
OC 2.17.	Processes and devices of pharmaceutical manufacturing, Course Project	3	Credit with the mark
OC 2.18.	Educational practice in botany	3	Credit with the mark
OC 2.19.	Drugs technology in pharmacy (industrial practice)	1,5	Credit with the mark
OC 2.20.	Technological practice (industrial practice)	1,5	Credit with the mark
OC 2.21.	Practice on the topic of the bachelor's degree qualification project	4,5	Credit with the mark
OC 2.22.	Performing of the bachelor's degree qualification project	9	Credit with the mark
OC 2.23.	Defense of the bachelor's degree qualification project		
Total for the cycle		***88,5***	
General amount of the obligatory program units		**179,5**	
Elective components of the Curriculum			
1. The cycle of general training			
Total for the cycle		***6***	
2. The cycle of professional training			
Elective components of EPP. Cycle 0100 «Industrial Pharmacy»			
EM 1.1.	Regulatory support of pharmaceutical industries	3	Examination
EM 1.2.	Basic of laboratory and functional diagnostics	3	Credit with the mark
EM 1.3.	Pharmacokinetics	3	Credit with the mark
EM 1.4.	Chemical and technology of medical compounds	7	Examination
EM 1.5.	Basic of clinical pharmacy	4	Credit with the mark
EM 1.6.	Basic of pharmacotherapy	4	Credit with the mark
EM 1.7.	Technology of drugs from natural raw materials and phytotherapy	5	Examination
EM 1.8.	Equipment and design of pharmaceutical industries	5	Examination
EM 1.9.	Management, marketing and pharmaceutical commodity science	4	Examination
EM 1.10.	Basics of emergency medical care	3,5	Credit with the mark
EM 1.11.	Physical methods of drugs’ analysis	4	Examination
EM 1.12.	Equipment and design of pharmaceutical industries, Course Project	3	Credit with the mark
Total for the cycle		***48,5***	

Cycle 0200 «Pharmacy»			
EM 2.1.	Laboratory and functional diagnostics and clinical pharmacy	6	Credit with the mark
EM 2.2.	Regulation and legislation of pharmaceutical companies	3	Examination
EM 2.3.	Technology of antibiotics and vitamins	3	Examination
EM 2.4.	Chemistry and technology of drugs	4	Examination
EM 2.5.	Design of chemical and pharmaceutical manufactures	5	Examination
EM 2.6.	Technology of galenical preparations	4	Examination
EM 2.7.	Pharmacology	6	Examination
EM 2.8.	Chemistry of cancerogens	3	Credit with the mark
EM 2.9.	Medical and pharmaceutical commodity science	4	Credit with the mark
EM 2.10.	Management and marketing in pharmacy	4	Credit with the mark
EM 2.11.	First aid	3,5	Credit with the mark
EM 2.12.	Design of chemical and pharmaceutical manufactures, Course Project	3	Credit with the mark
Total for the cycle		***48,5***	
General amount of the elective program units		**75**	
Elective components of others EPP			
Total		***6***	
General amount of the elective program units		**60,5**	
GENERAL AMOUNT OF THE SYTLLABUS		**240**	

Table 4

The list of educational components of EPP "Pharmacy, Industrial Pharmacy" for the second (master's) degree of higher education in Lviv Polytechnic National University

Subject matter code	Curriculum unit (subject matter, course projects, practical training, qualification project)	Number of credits	Form of final control
1	2	3	4
Obligatory EPP component for specialty			
1. The cycle of general training			
OC 1.1.	Economics of chemical and pharmaceutical enterprises	4	Examination
Total for the cycle		***4***	
2. The cycle of professional training			
OC 2.1.	Modeling and design of chemical and pharmaceutical enterprises in the GMP system	6	Examination
OC 2.2.	Scientific aspects of ecology of chemical and pharmaceutical industries	4	Credit with the mark
OC 2.3.	Scientific aspects of technology of veterinary and biomedical drugs	7	Credit with the mark
OC 2.4.	Industrial technology of pharmaceutical manufacturing, part 1	6	Examination
OC 2.5.	Occupational and civil safety	3	Credit with the mark
OC 2.6.	Industrial equipment of chemical and pharmaceutical enterprises	3	Examination
OC 2.7.	Modeling and design of chemical and pharmaceutical enterprises in the GMP system, Course Project	3	Credit with the mark
OC 2.8.	Practice on the topic of the master's degree qualification project	9	Credit with the mark
OC 2.9.	Performing of the master's degree qualification project	16,5	
OC 2.10.	Defense of the master's degree qualification project	4,5	
Total for the cycle		***62***	
General amount of the obligatory program units		**66**	
Elective components of the Curriculum			

1. *The cycle of general training*			
Disciplines of the student's choice			
Total for the cycle		***3***	Credit with the mark
2. *The cycle of professional training*			
Elective components of cycle 01 «Industrial Pharmacy»			
EM 1.1.	Quality control of medicines	3	Credit with the mark
EM 1.2.	Industrial technology of pharmaceutical manufacturing, part 2	4	Examination
EM 1.3.	Technology of biologically active substances, biomedical polymers and nanostructures	5	Credit with the mark
EM 1.4.	Technology and application of medical cosmetics	4	Credit with the mark
Total for the cycle		***16***	
Elective components of cycle 02 «Pharmacy»			
EM 2.1.	Clinical Pharmacy	4	Credit with the mark
EM 2.2.	Scientific aspects of biopharmacy	4	Credit with the mark
EM 2.3.	Quality assessment of medicines	4	Examination
EM 2.4.	Pharmacotherapy	4	Credit with the mark
Total for the cycle		***16***	
Disciplines of the student's choice			
Total for the cycle		***5***	Credit with the mark
General amount of the elective program units		**24**	
GENERAL AMOUNT OF THE SYTLLABUS		**90**	

Table 5

The list of educational components of educational scientific program (ESP)
"Pharmacy, Industrial Pharmacy"
for the second (master’s) degree of higher education in Lviv Polytechnic National University

Subject matter code	Curriculum unit (subject matter, course projects, practical training, qualification project)	Number of credits	Form of final control
1	2	3	4
Obligatory ESP component for specialty			
1. The cycle of general training			
OC 1.1.	Economics of chemical and pharmaceutical enterprises	4	Examination
Total for the cycle		***4***	
2. The cycle of professional training			
OC2.1.	Modeling and design of chemical and pharmaceutical enterprises in the GMP system	6	Examination
OC 2.2.	Scientific aspects of ecology of chemical and pharmaceutical industries	4	Credit with the mark
OC 2.3.	Scientific aspects of technology of veterinary and biomedical drugs	7	Credit with the mark
OC 2.4.	Industrial technology of pharmaceutical manufacturing, part 1	6	Examination
OC 2.5.	Occupational and civil safety	3	Credit with the mark
OC 2.6.	Industrial equipment of chemical and pharmaceutical enterprises	3	Examination
OC 2.7.	Clinical and pharmaceutical aspects of drugs use (special course, part 3)	3	Examination
OC 2.8	Research and seminars on the subject	9	Credit with the mark
OC 2.9	Fundamentals of fine organic synthesis (special course, part 2)	3	Examination
OC 2.10	Basic of pharmaceutical biochemistry (special course, part 1)	3	Examination

OC 2.11	Workshop on preparing of scientific publications, conference materials and presentations of scientific reports	4,5	Credit with the mark
OC 2.12	Modeling and design of chemical and pharmaceutical enterprises in the GMP system, Course Project	3	Credit with the mark
OC 2.13	Educational and research practice	6	Credit with the mark
OC 2.14	Practice on the topic of the master's degree qualification project	12	Credit with the mark
OC 2.15	Performing of the master's degree qualification project	18	
OC2.16	Defense of the master's degree qualification project	1,5	
Total for the cycle		**92**	
General amount of the obligatory program units		**96**	
Elective components of the Curriculum			
1. The cycle of general training			
Disciplines of the student's choice			
Total for the cycle		***3***	Credit with the mark
2. The cycle of professional training			
Elective components of cycle 01 «Industrial Pharmacy»			
EM 1.1.	Quality control of medicines	3	Credit with the mark
EM 1.2.	Industrial technology of pharmaceutical manufacturing, part 2	4	Credit with the mark
EM 1.3.	Technology of biologically active substances, biomedical polymers and nanostructures	5	Credit with the mark
EM 1.4.	Technology and application of medical cosmetics	4	Credit with the mark
Total for the cycle		**16**	
Elective components of cycle 02 «Pharmacy»			
EM 2.1.	Clinical Pharmacy	4	Credit with the mark
EM 2.2.	Scientific aspects of biopharmacy	4	Credit with the mark
EM 2.3.	Quality assessment of medicines	4	Examination
EM 2.4.	Pharmacotherapy	4	Credit with the mark
Total for the cycle		***16***	
Disciplines of the student's choice			
Total for the cycle		***5***	Credit with the mark
General amount of the elective program units		**24**	
GENERAL AMOUNT OF THE SYTLLABUS		**120**	

Analysis of educational programs of specialty 226 "Pharmacy, industrial pharmacy", which are approved in Lviv Polytechnic National University and Danylo Halytsky Lviv National Medical University for specialists training testifies to a significant amount of common educational components, especially the pharmaceutical direction, that is considered as a positive sign for the creation of future Education Standards. However, it should be noted, that the OPP for the first (bachelor's) degree of higher education in Lviv Polytechnic National University includes a large number of disciplines of the cycle of general and professional training such as Higher Mathematics, Physics, Engineering and Computer Graphics, Processes and devices of pharmaceutical manufacturing, etc., are necessary and considered as fundamentals for further components of the educational program that directly form a specialist in the industrial drugs technology and the acquired competencies allow applicants to successfully perform and defense the bachelor's degree qualification project. The development and approved of the Education Standard in the specialty 226 "Pharmacy, Industrial Pharmacy" is extremely important, because will contribute to a single format and content of the list of components of educational programs, unify the form of final certification, and will guarantee competitiveness in the labor market for all applicants of higher education. To find a compromise to solve this problem, we have started cooperation with five HEIs, which

actually train specialists in the specialty "Industrial Pharmacy", to develop and create a database of questions for the STEP 2 test exam.

At the first stage of our joint research, the lists of disciplines in different HEIs were collected and analyzed, and the structure of the integrated test exam of SSQE STEP 2 for direction "Industrial Pharmacy" was formed (Table 6).

Table 6

Courses for professional training of specialists of the second (master’s) degree of higher education in the specialty 226 "Pharmacy, industrial pharmacy" for direction "Industrial Pharmacy"

Lviv Polytechnic National University	Ukrainian State University of Chemical Technology	Odesa Polytechnic State University	Kyiv National University of Technologies and Design	The structure of the integrated test exam SSQE KROC 2, axis 2
Modeling and design of chemical and pharmaceutical enterprises in the GMP system	Automated process control systems	Biotechnology in pharmacy and cosmetology	Industrial biotechnology of drugs	Industrial biotechnology of drugs
Scientific aspects of ecology of chemical and pharmaceutical industries	Validation process	Standardization of drugs	Validation of technological process and analytical methods	Validation process
Scientific aspects of technology of veterinary and biomedical drugs	Modern pharmaceutical technologies	Drugs technology in pharmacy	Technologies of active pharmaceutical ingredients	Modern pharmaceutical technologies
Industrial technology of pharmaceutical manufacturing	Special equipment and design of chemical and pharmaceutical industries	Cosmetic chemistry	Special equipment and design of chemical and pharmaceutical industries	Special equipment and design of chemical and pharmaceutical industries
Industrial equipment of chemical and pharmaceutical enterprises	Pharmaceutical development of drugs	Basic of scientific research in the chemical and pharmaceutical branch	Pharmaceutical development of drugs	Pharmaceutical development of drugs
Quality control of medicines		Automated systems and information technology in pharmacy	Pharmaceutical quality system and quality control of medicines	Pharmaceutical quality system and quality control of medicines
Technology of biologically active substances, biomedical polymers and nanostructures				

Further research will concern the development of a database of questions for the SSQE STEP 1 and STEP 2 exams It would be much easier to create such a database if the Education Standard will be approved and available. However, the elaboration of such a Standard must be preceded by the division of specialty 226 "Pharmacy, Industrial Pharmacy" into two separate ones - "Pharmacy" and "Industrial Pharmacy", which must belong to the same field of knowledge 22 "Health care".

Conclusions

1. Ukraine as a state, according to the Constitution and other regulations, undertakes a number of very important obligations regarding health care, especially, ensure monitoring the quality of educational services and the educational process in higher education institutions.

2. The specialty "Industrial Pharmacy" should be saved in the field of knowledge 22 "Health Care", as its transfer to the branch of knowledge 18 "Manufacturing and Technology" levels the results of specialists training, threatens the existence of the specialty and makes it impossible for the functioning pharmaceutical industry of Ukraine.

3. As a result of the transfer of specialty "Industrial Pharmacy" from the field of knowledge 22 "Health Care" to the branch of knowledge 18 "Manufacturing and Technology", obstacles will be created for the research realization, development, and implementation of new medicines and generic drugs.

4. It is extremely important to save the specialty "Industrial Pharmacy" in the field of knowledge 22 "Health Care". This is the only possible way to guarantee the quality of the drugs according to the Complex of Good Pharmaceutical Practices (GMP, GLP, GCP, GDP, GPP, GSP, GEP, GPEP) at all stages of its promotion from manufacturer to the patient, that will facilitate scientific research and development of new safe, effective, high-quality medicines, and also will allow providing training of highly skilled specialists for pharmaceutical branch.

5. Traditionally, specialists for pharmaceutical manufacturing were trained mostly at the higher education institutions, which were subordinated to the Ministry of Education and Science of Ukraine, and those for pharmacies and hospital pharmacies were trained at the higher education institutions, subordinated to the Ministry of Health of Ukraine. However, with the introduction in 2016 of a single specialty 226 "Pharmacy", and in 2017 with the renaming of the specialty to 226 "Pharmacy, Industrial Pharmacy", in the higher education institutions of different subordination (Ministry of Health of Ukraine and Ministry of Education and Science of Ukraine) is training professionals for the pharmaceutical industry. The only significant difference is the quality control of student training. In the higher education institutions, subordinated to the Ministry of Health of Ukraine, the assessment system STEP 1, 2, 3 exams has been introduced. In the higher education institutions subordinated to the Ministry of Education and Science of Ukraine, students perform and defense bachelors or master's qualification projects as a form of certification of applicants of the degree of higher education. We completely agree with the requirement to move to a system for assessing the quality of knowledge in accordance with the STEP 1 and STEP 2 exam systems and it is important to ensure a smooth, adequate transition to this system and coordinate the actions of higher education institutions, which found themselves in a similar situation.

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Chapter 4. CHANGES IN THE ELECTROPHYSICAL PROPERTIES OF NATURAL DRINKING WATER IN ITS EXPERIMENTAL COHERENCE WITH DIFFERENT POLARITY AND DEGREE

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Abstract. *The article presents experimental studies results on electrophysical properties changes of natural drinking water after its coherence according to the results of Kirlian photography. The study issue is relevant to connection between health water properties and its coherence. The coherent state determines nonlinear effects occurrence, which leads to the water molecules total response to Kirlian irradiation. Gas–discharge glow of 50 drops of each water sample was received on an X-ray film. Water coherence was carried out by using a quantum teleportation system. A coherent state of water was created with different spin directions (right and left) and the degree of coherence (1-3). Computer processing of the Kirlian water droplets photographs was carried out by building brightness histograms. The results were compared with the previously developed by us criteria for median values and the medians difference of the parameters of 12 histogram subranges for experimental standard water samples – distilled, tap water, from natural drinking sources outside and from the territories of monasteries. The indicators differences degree was analyzed according to the Euclidean distances for medians values and differences in medians with each typical water sample. Distilled water was used as an incoherent standard. Various changes patterns of the studied values were obtained for the left and right water polarization of the samples and with an increase in its degree. A step–by–step change in the electrophysical properties of the control samples was observed for both types of their coherence with a smaller interval for the left direction. Levorotatory coherent water acquired new stable properties already during the first impact degree. The established experimental Kirlian characteristics can be used as a water coherence express-test.*

Keywords: *coherent water, Kirlian photography, X-ray film.*

Introduction

Until recently, the role of electronically excited states was not taken into account. Nevertheless, the dependence on these states of the biological water properties is known. On the basis of quantum electrodynamics, it was proved that liquid water is a coalition, a set of coherent domains. If the domain is in the lowest energy state (the main state), all electrons are firmly connected. So for water ionization it is required that it receives an energy pulse that corresponds to a soft x–ray radiation. Therefore, in the excited state, many electrons are almost free, and low

energy is needed for the electrons to become completely free. In the incoherent state, the water molecule cannot act as electron donor recover, and coherent water is a good reducing agent.

We earlier reported on the use of Kirlianphotography, as a method based on the effect of gas–discharge Kirlian glow around the water droplets, to assess its health properties. Today, some ideas are being developed about connections of the latter with the coherent water state.

Informative signs of images of gas-discharge radiation of water droplets from different natural sources on an X-ray filmwere analyzed, previously scanned. The samples of Kirlian (KI) were formed out of more than 900 drops of typical water samples (TW): distilled – TW 1, plumbing – TW 2, from natural sources – TW 3 and from the territories of monasteries – TW 4).

Since the method of classical Kirlian photography is easy to perform with high informativeness and high sensitivity to external influences, it is advisable to study possibilities of its use in express assessment of water samples coherence condition according to theuse results of Kirlian photography method.

The purpose of the research was to study the changes in the electrophysical properties of experimental coherent natural drinking water at different degrees of opposite polarization in comparison with its control samples.

Material and research methods. Natural drinking water (CDW) was used as a control sample. Its coherenization was performed by using the developed quantum teleportation system described in, with the help of which at a distance of about 500 km (from Kiev to the city of Dnipro) a coherent state of DW with different spin orientation (right and left) and degree of coherencewas created. (1, 2, 3).

A special chip represented the element of a singlent pair with a translation symmetry in the form of a metal plate, 5x5 mm, which was attached to the outside of a glass cup, which was filledwith packed, natural drinking water. The water volume for research was taken in the amount of 50 ml. At the beginning, a L–chip (left–sided orientation of spins) was taken to activate the water, and then the R–chip, which was attached to another cup with the same packaged water. After filling the initial DW into the cups, using measurements of water physical properties, it was observed in the dynamics of "guidance" using chips of the water coherent state.

This method lies in the fact that the introduction of water into the coherent state occurs through spin saturation of water, which is carried out before the process of Kirlian irradiation. Spin saturation is achieved by contacting water with a chip inducer placed on water tanks, and the exposure to water occurs continuously to the irradiation process. As a result of the spin saturation and interaction of the spin–grid (spin–molecule), the water molecular structure begins to oscillatrate with one frequency and with one phase, which leads to the coherent state.

The coherent state determines nonlinear effects appearance, which leads to the total response of water molecules on Kirlian irradiation.

Kirlian photographing was carried out with 50 drops of control and experimental water samples. An X–ray film was used, an experimental device with a console for liquid–phase objects was developed with participation of the "National University of Ukraine's Health Protection. L. Schupika" (Kiev) and NTU "Dniprovskaya Polytechnica" (Dnipro, Ukraine).

To study water properties histograms of image brightness were built, which allow to identify geometric and bright–light (photometric) image parameters. Specific radiation features are highlighted for a specific water type by analyzation and parametrization of brightness histograms. Averaging specific radiation signs for samples within one water type based on calculations of medians and their differences. From the point of view of mathematical statistics it allowed us to implement a steady (robust) approach to the processing of experimental data, since the median is an experimental assessment of the mathematical expectation, which ensures resistance torandom emission and misses.

As the most likely value of the column height, the magnitude of the median was used, calculated for the corresponding sample of images. The further classification procedure was based on the use of metric – the Euclidean distance between the heights of the corresponding histogram columns. As an additional criterion, the difference of histogram column elements in adjacent

intervals were used to compare water samples. They were calculated by subtracting the magnitudes from the subsequent height – the height of the previous interval. The physical meaning of the application of this indicator consists in the possibility of tracking dynamic changes in the brightness indicators from one interval to another histogram interval.

Maximum amplitude in the histogram of brightness corresponds to the background of the X–ray film. For distilled water, without impurities, this peak turns out to be the only extremum for the graph of the image brightness histogram. It is the worst version from the point of view of its quantum and biological properties and can be used as a standard of non–coherent fluid. Its molecular structure is constructed in such a way that it cannot act as a source of free charge carriers. Itis manifested in a weak streamer crown of the luminescence around the drop and the smallest luminescence intensity, compared with other types of water.

For water with impuritiesinclusions, the histogram is multimodal. For a sample of tap water, a substantially pronounced grainy structure in the inner circle of the glow corresponding to the drop itself is inherent. Water samples from natural sources have higher biological indicators of the gas–discharge glow criteria. According to the results of the previously conducted experimental data, together with professor Kurikom M.V., in biological properties the most highly functional was monastic water. Figure 1 shows examples of Kirlian images of different water samples.

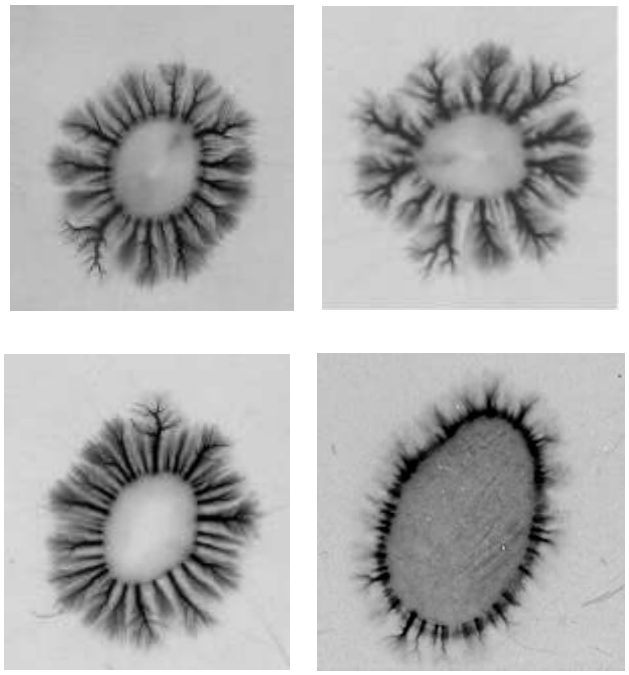

Fig. 1. Kirlian images examples of various water samples

We conducted computer processing of Kirlian images of control samples of natural drinking water (CDW) before and after coherence. The results were compared with the magnitudes of the median and the difference between the parameters for experimental typal water developed by our early criteria for experimental typal water – distilled (type 1), plumbing (type 2), from natural drinking sources outside the territories of monasteries (type 3), from natural drinking sources on the territory of Kiev Pechersk Lavra (type 4). Degree of differences in the indicators was analyzed by the Euclidean distances for median values (EDM) and median differences (EDDM) when compared with each sample of typal water (TW 1–4).

The obtained results and their discussion. Table 1 presents the analysis results of brightness histograms for control and experimental samples of coherent drinking water for different coherence types and degrees.

Note: EDM – Euclidian median, EDDM – Euclidian distance median difference, CDW–control water drinking, CHD – coherent water on drinking water, (+) – right–sided coherence polarization, (–) – left–sided, (1–3) – coherence degrees.

To eliminate the effect of the initial characteristics of the control samples on the characteristics of coherent water samples, the values of the control samples were subtracted from the latter values, which is presented in Table 2.

Table 1

Data of brightness histograms for control and experimental samples of coherent drinking water for different coherence types and degrees

Water samples, pH	EDM 1 type	EDM 2 type	EDM 3 type	EDM 4 type	EDDM 1 type	EDDM 2 type	EDDM 3 type	EDDM 4 type
CDW (+1) pH 4,8	49125	54103	53974	45404	48127	53177	52370	47036
CDW(+2) pH 4,7	35131	33667	34661	33413	38316	38539	38740	39502
CDW(+3) pH 4,6	43717	50367	52534	41264	60884	67972	69808	56605
CHD (+1) pH 4,99	60565	58376	56928	58613	86096	81763	81309	88793
CHD (+2) pH 4,76	57203	54211	52714	55666	84356	79472	79137	87409
CHD (+3) pH 4,79	43953	51586	53270	40457	56581	64559	66147	51715
CDW (–1) pH 4,6	59904	69156	70951	56964	80328	88529	90449	74956
CDW (–2) pH 4,5	69146	71361	69267	67152	95668	95728	93303	97978
CDW (–3) pH 4,7	34597	37580	38233	31667	39799	44933	44567	38920
CHD (–1) pH 4,61	42313	43143	41353	41970	49435	49526	45320	54186
CHD (–2) pH 4.42	46163	50623	49530	43896	50899	53843	51524	52891
CHD (–3) pH 4,76	33351	35172	36126	33512	37595	41597	41200	38104

Table 2

Differences between histogram indicators of DW control samples brightness and the corresponding samples of CHD of different degrees and polarization compared with typical water samples

The difference between the CDW and the CHD	pH	EDM 1 type	EDM 2 type	EDM 3 type	EDM 4 type	EDDM 1 type	EDDM 2 type	EDDM 3 type	EDDM 4 type
For +1	0,18	–11439	–4274	–2955	–13209	–37969	–28586	–28940	–41757
For +2	0,11	–22073	–20544	–18052	–22253	–46040	–40932	–40397	–47907
For +3	0,24	–236	–1219	–737	807	4304	3413	3661	4890
For –1	0,05	17592	26012	29599	14995	30892	39003	45128	20771
For –2	0,09	22983	20737	19736	23257	44769	41885	41779	45087
For –3	–0,09	1247	2408	2107	–1844	2205	3335	3367	816

Note: the same

Table 3 presents data of comparative analysis of brightness histogram of control and experimental samples of CHD of both types with samples of typical water.

Table 3

Comparative analysis of EDM and EDDM brightness histogram of the control and experimental samples of CHD with samples of typical water

Samples	EDM 1 type	EDM 2 type	EDM 3 type	EDM 4 type	EDDM 1 type	EDDM 2 type	EDDM 3 type	EDDM 4 type
CDW (+1) and (+2)	13995	20435	19312	11990	9810	14638	13629	7535
CDW (+2) and (+3)	–8587	–16699	–17872	–7850	–22568	–29433	–31067	–17104
CDW (+1) and (+3)	5408	3736	1440	4140	–12758	–14795	–17438	7535
CHD (+1) and (+2)	3361	4165	4215	2946	1739	2292	2172	1385
CHD (+2) and (+3)	13250	2626	–557	15210	27776	14912	12991	35693
CHD (+1) and (+3)	56169	6791	3658	18156	29515	17204	15163	37078
CDW (–1) and (–2)	–9242	–2205	1685	–10188	–15341	–7198	–2854	–23021
CDW (–2) and (–3)	34549	33780	31034	35485	55869	50795	48736	59058
CDW (–1) and (–3)	25307	31575	32719	25297	40528	43597	45882	36037
CHD (–1) and (–2)	–3851	–7480	–8178	–1926	–1464	–4316	–6203	1295
CHD (–2) and (–3)	12813	15451	13405	10384	13305	12245	10324	14787
CHD (–1) and (–3)	8962	7971	5227	8458	11841	7929	4121	16082

The change in the degrees (+) coherence of natural drinking water in the magnitude of the EDM in comparison with TW 2 and TW 3 demonstrated similar trends among them, but different from TW 1 and TW 4. There are greater differences of all TW indicators in CHD (+2) and the smaller – in CHD (+3), compared with CHD (+2), but higher than of Water CHD (+1), the wave process is observed. Namely, compared with TW 2 and TW 3, as well as with TW 4, but changes in magnitude unlike TW 4 "delay". The latter is natural, because TW 4 water initially has ahighlyordered structure.

The difference in EDM in CHD indicators of dextrorotatory polarization with typal water at the 2nd degree is less than at the 1st degree. Large differences were when compared with tap water, which is explained as the most structurally different from other typical water as a result of technogenic effect. Smaller differences were when compared with monastic water (TW 4), as the most structured and initially coherent. Moreover, the difference between the control samples was significantly higher in EDM with all kinds of typal water.

In contrast to the dextrorotatory (+) coherence of natural drinking water, with left–side (–) coherence, there is a clear increase in the differences from TW 2 (tap water) and TW 3 (natural)

according to the degree of coherence and, less – from TW 1, minimally – from TW 4. At the same time, the control samples of them, on the contrary, were as different as possible on the EDM with the latter and slightly from TW 2 and TW 3.

Thus, the levorotatory coherence water prepared on natural drinking water, with the initially determined coherence, with a smaller (–) effect on it acquires properties, which more significantly distinguish it from the tap and typical natural water. At the same time, the 1st and 2nd degrees differ a little among themselves. Namely, having a smaller impact, it reveals quite stable other physical properties, as with (+) polarization. It changes in parameters of the latter, in contrast to changes in CHD samples (–), did not reveal the distinctiveness from more coherent TW monastic water.

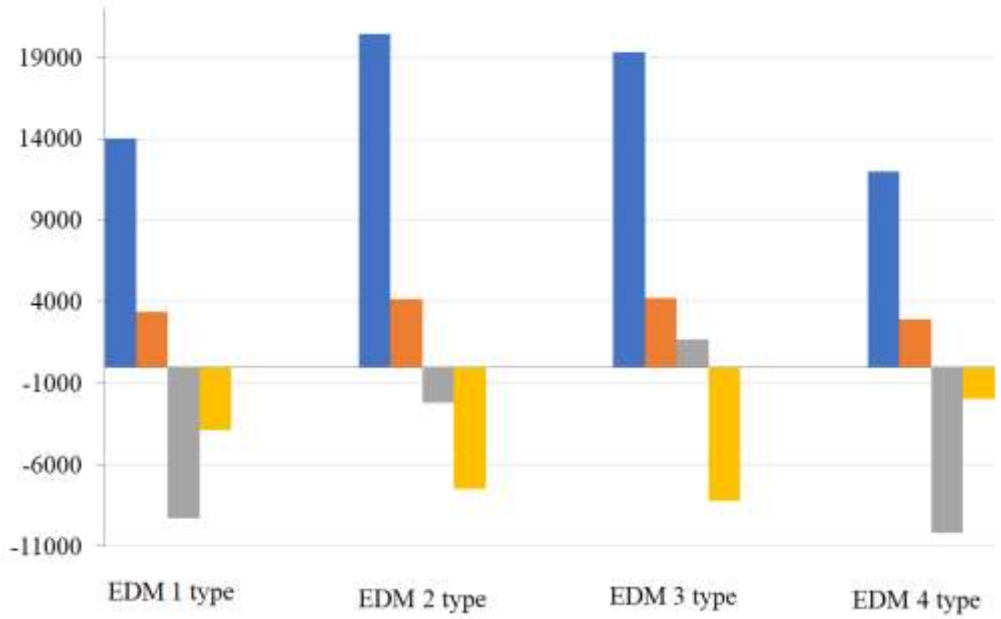

Chart 1. The indicators difference in the EDM control and experimental samples of CHD with typal water (1 column – between CDW (+1) and CDW (+2); 2 column – between CHD (+1) and CHD (+2); 3 column – between CDW (–1) and (–2); 4 column – between CHD (–1) and CHD (–2)

With the 3rd degree (+) of coherence the differences from CHD (+2) revealed the same as in CHD (–2), the differences between monastery water from TW2 and TW3. Differences on EDM with TW between samples (–) CHD 2nd and 3–step degrees were insignificant. Between the samples of CHD (+2) and CHD (+3) were explicit withTW4, with the smallest differences from it. At the same time, in control samples of the CDW, the opposite patterns were observed in them, which demonstrates competence of the water coherence method.

When analyzing the EDM values between the samples of CHD (–2) and CHD (–3) with typal water, there was a decrease in differences with all typal water. They were 2 times more than among control samples. Water coherence changes them, making them more similar. Smaller differences between CHD (–3) and CHD (–2) were on the EDM with monastic water, but exceeded the difference between the parameters of CHD (–1) and CHD (–2). There is a step–by–step change in the physical properties of drinking natural water at both types of coherence, with a smaller interval at (–) polarization.

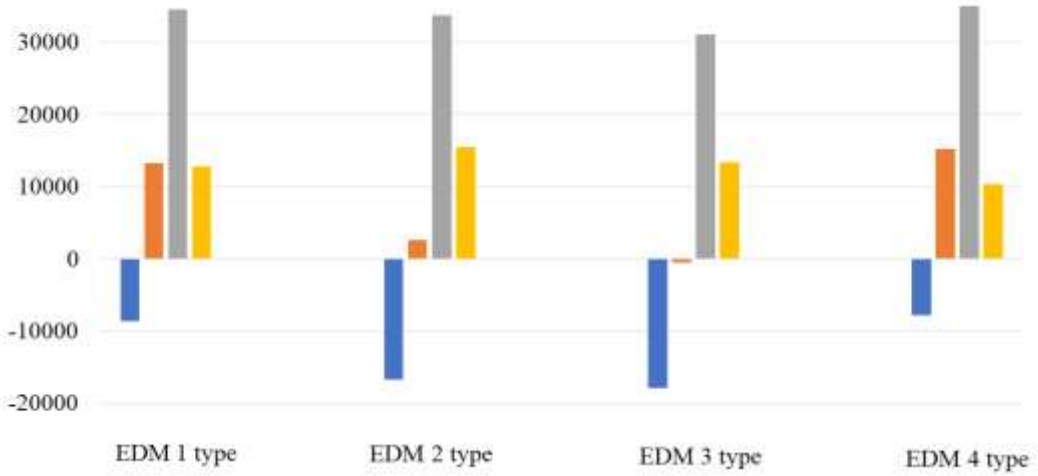

Diagram 2. The difference in indicators of the EDM of control and experimental samples of CHD with typal water (1 column – between CDW (+2) and CDW (+3); 2 column – between CHD (+2) and CHD (+3); 3 column – between CDW (–2) and (–3); 4 column – between CHD (–2) and CHD (–3)

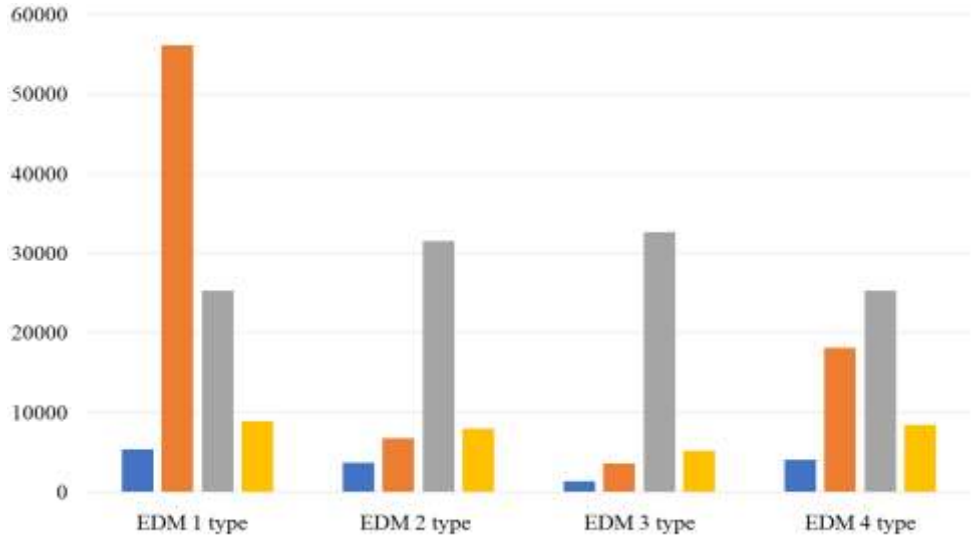

Diagram 3. Differences of indicators of the EDM of control and experimental samples of CHD with typal water (1 column – between CDW (+1) and CDW (+3); 2 column– between CHD (+1) and CHD (+3); 3 column– between CDW (–1) and (–3); 4 column – between CHD (–1) and CHD (–3)

As a result, while comparing the 1st and the 3rd degrees of samples (+) CHD of water, there are pronounced differences in their EDM with TW1 (distilled) and TW4 (monastic) water, compared with CHD (+2). Differences with TW1 – can be explained, since the distilled water is not coherent and during coherenization process it will differ less from the initial. CDW as initially having a certain coherence degree and polarity, already at (+) the 1st degree of coherence with our method, is easily rebuilt and acquired certain resistant electrophysical features that distinguish it from typical water 2 (tap water) and 3 (natural sources outside monasteries). With the 3rd degree of coherence, they are moderately and little differ from (+1) degrees, respectively. Control samples of DW for these degrees of coherence are close to each other by moderate differences of their EDM with typal water.

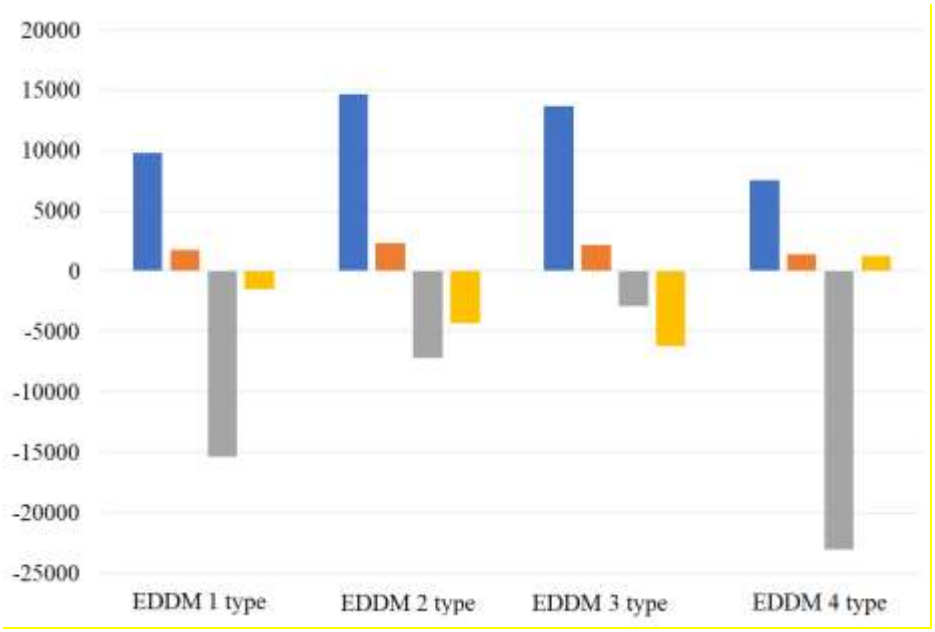

Diagram 4. Differences of EDDM indicators of control and experimental samples of CHD with typal water (1 column – between CDW (+1) and CDW (+2); 2 column – between CHD (+1) and CHD (+2); 3 column – between CDW (–1) and (–2); 4 column – between CHD (–1) and CHD (–2) (Table 3)

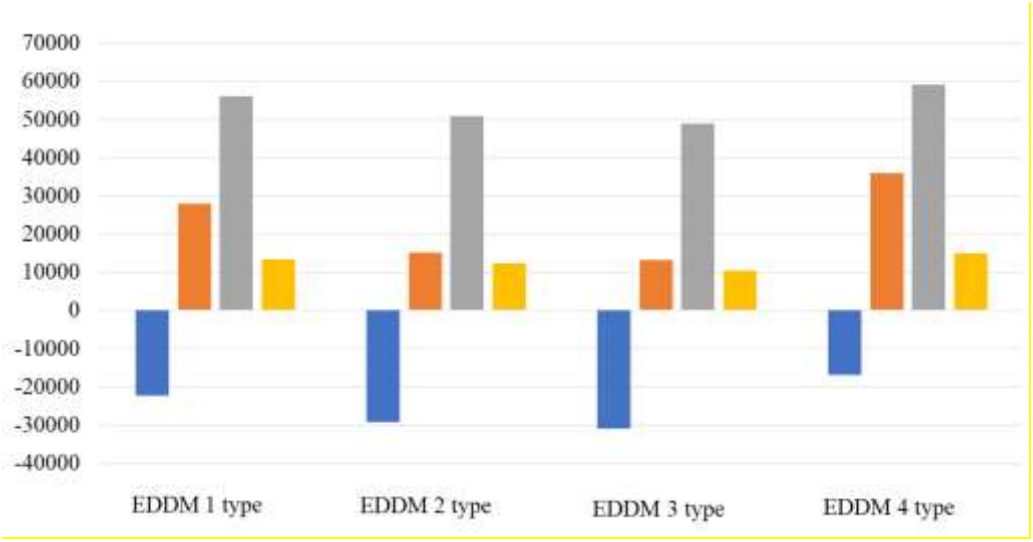

Diagram 5. Differences of EDDM indicators of control and experimental samples of CHD with typal water (1 column – between CDW (+2) and CDW (+3); 2 column – between CHD (+2) and CHD (+3); 3 column – between CDW (–2) and (–3); 4 column – between CHD (–2) and CHD (–3)

During levorotatory coherence as well as during (+) the smallest differences between the degrees were with respect to TW3 (natural sources outside monasteries). It is connected to a certain extent with preserving of natural water initial structural features. At the same time, at (–) coherence stable differences from the initial nature of DW and the approach to the monastic water was observed already at the 1st degree of coherence, while these differences decreased in the subsequent degrees. Differences from other TW were more demonstrative as compared with the 1st and 2nd degrees of CHD water. With (+) coherence, they are more pronounced when comparing the 1st and 2nd degrees of CHD water with the 3rd.

There are similar to EDM consistent patterns – are of minor differences, but smaller between EDDM indicators with all the TW between the samples of the 1st and 2nd degrees of CHD water.

At (–) polarization in CHD (–2) water differences with TW 2 and TW 3 more than at CHD (–1), with TW 1 and TW 4 they are not significant.

From the data similar patterns were observed. They were obtained while analyzing the EDM values. In particular, the differences between the 2nd and 3rd degrees of CHD (+) in EDDM with TW1 and TW4 are pronounced. The differences in EDDM with TW 2 and TW 3 are 2 times less, which confirm the different structural and electrical properties of water from natural sources outside and on the monasteries territory. Differences of the values at (–) coherence between the 2nd and 3rd degrees of typal water, as well as of EDM, were less demonstrative. Also we revealed parameters differences in the samples of the control water taken from one source.

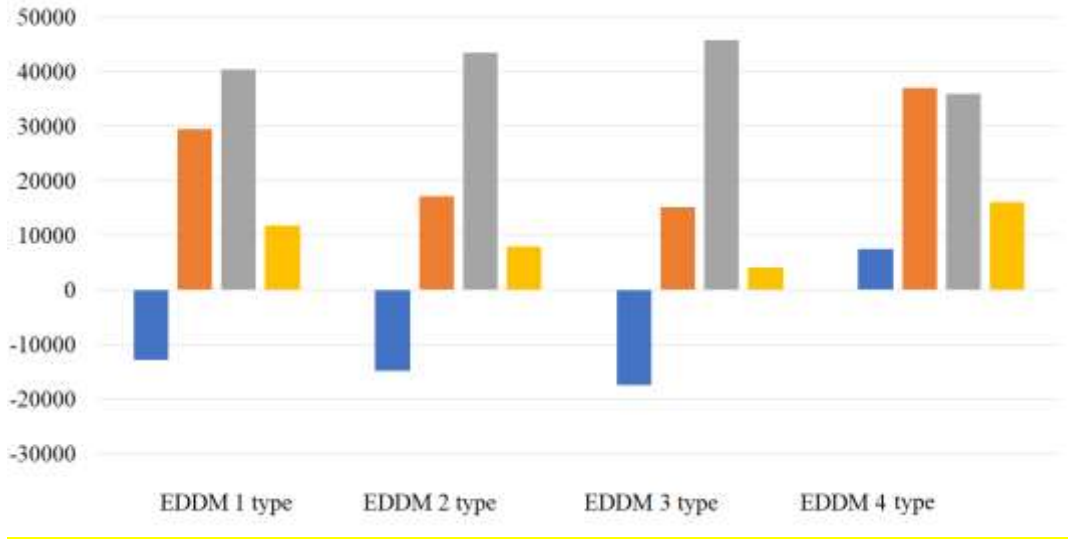

Diagram 6. Differences of EDDM indicators of the control and experimental samples of CHD with typal water (1 column – between CDW (+1) and CDW (+3); 2 of column – between CHD (+1) and CHD (+3); 3column – between CDW (–1) and (–3); 4 column – between CHD (–1) and CHD (–3)

As in the ECM indicators, there are significant differences on the comparative analysis diagram of EDDM during the (+)of drinking water between the 1st and 3rd degree with typical TW 1 and TW 4 samples and two fewer differences with TW 2 and TW 3. The differences between natural and monastic monastery are discovered again. At (–) coherenceof CDW, as well as according to the results of EDM, the differences in degrees were the smallest with TW 3. However, in EDDM, we obviously observe the maximum differences with TW 4, compared with the less pronounced on EDM. Thus, when assessing the type and degree of water coherence, it is necessary to analyze both parameters of the brightness histogram of Kirlian photography of water.

Also, as in EDM, with (+) coherence, more explicit differences between the quantities were observed when compared with the 1st and 2nd degrees of CHD of water with the 3rd one. At (–) coherence, the resistant magnitudes approximation to monastic water was observed at the 1st degree of coherence, with the differences decrease in the subsequent degrees. Differences from other TW were more demonstrative also when compared with the 1st and 2nd degrees of CHD water at (–) polarization and the 1st and 2nd degrees of CHD of water with the 3rd one.

Conclusion

1. Kirlian photography of water is a rather informative method for evaluating its electrophysical properties.

2. Method of computer analysis of the Kirlian images, applied by us for allocation of 4 classes of typal water, characterized by the coherence degree, with the highest coherence degree from monastic sources, can be used as an express method to estimate coherence of water prototypes.

3. In the research of the electrophysical water properties, a comparative analysis with control water samples is necessary, including water sampleswhich were taken from one water source.

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Chapter 5. EFFICIENCY OF LONG-TERM PASSIVE THERAPY FOR RECOVERY OF LIMB FUNCTION AFTER TOTAL KNEE ARTHROPLASTY

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Abstract. *Total knee arthroplasty is considered a radical and effective method of treating osteoarthritis of the knee. The meta-analysis of scientific researches regarding the efficiency of long-term rehabilitation after total knee arthroplasty is carried out and supplemented. The complex program for physical rehabilitation in combination with the Artromot Active-K device in the long-term rehabilitation period after total knee arthroplasty is proposed. The effectiveness of the developed set of physical exercises in combination with apparatus methodology and their use in restoring the functions of the limb after endoprosthetics, which significantly reduces the intensity of pain, increases of the knee joint mobility, expands the motor mode of patients, is proved in practice. The results of the scientific researchare of great practical importance for the improvement of the long-term rehabilitation system for persons after total knee arthroplasty.*

Keywords: *osteoarthritis, gonarthrosis, knee joint, total knee arthroplasty, physical rehabilitation, physiotherapy exercises.*

Introduction

The knee joint (KJ) in everyday life experiences enormous stress, most often several times the body weight. Such an excessive strain per unit area of the articular cartilage is a strong negative factor resulting in its degeneration. When descending the stairs in the KJ on the top line, the peak strain on the articular cartilage reaches 346% of the body weight, when climbing stairs – 316%, when getting up from a chair – 246%, when sitting on a chair – 225%, when standing on one leg – 259%, when standing on two legs, the effect on the cartilage of the joint is 7% more than the body weight (Ankin, 2012).

Gonarthrosis (deforming arthrosis, osteoarthrosis (OA), osteoarthritis, degenerative arthritis) is a polyetiological degenerative-dystrophic disease of the KJ, characterized by damage to the hyaline cartilage, followed by the formation of osteocartilaginous growths, deformation. Disintegration of joint cartilage is the main pathological manifestation of OA. Along with the destruction of articular cartilage in case of degenerative-dystrophic diseases, other components of the joint are also involved: subchondral bones, synovial membrane, joint ligaments and capsule, periarticular muscles. OA, also known as degenerative articular disease of the joints, is usually a result of wear and tear and possible loss of articular cartilage. More common of older women and men (Arden, & Nevitt, 2006).

Arthrosis of the KJ can be divided into two types, primary and secondary. Primary osteoarthrosis is joint degeneration for no apparent underlying reason. Secondary osteoarthrosis is a consequence of either an abnormal concentration of force in the joint, both for post-traumatic reasons, and abnormal articular cartilage, such as rheumatoid arthritis (RA). Osteoarthrosis is usually a progressive disease that can result in disability over time. The intensity of clinical symptoms may differ from person to person. However, they become more severe, more frequent and debilitating over time. The rate of progression also varies for each person. Common clinical symptoms include knee pain that gradually begins and increases when moving, stiffness and swelling of the knee, pain after prolonged sitting or rest, and increasing pain over time (Cyrus Cooper, Javaid, & Arden, 2014).

Osteoarthrosis of the KJ accounts for 24.7 to 54.5% of diseases of large joints. The progression of degenerative-dystrophic diseases of the KJ within 12-15 years results in disability often even of working age. Osteoarthrosis of the KJ in 10-21% of cases causes a decrease in working capacity and disability. Disabled is 1 in 100 patients suffering from diseases of the locomotorium; the most severe course is characterized by KJ osteoarthrosis. A significant decrease in the quality of life of disabled persons is associated with joint pain, reduced mobility and loss of the ability to look after themselves (Dubrovin,2003).

The main reason for the development of OA is an imbalance of the processes of destruction of cartilage and its recovery due to various endogenous and exogenous factors, such as joint injury, ratio distortion of surfaces as a result of congenital, traumatic reasons; metabolic and endocrine disorders, overweight. As a result, the normal strain becomes excessive and causes degeneration of the articular cartilage, resulting in deforming arthrosis and aseptic joint inflammation. Inflammatory processes of an infectious and non-communicable nature, dystrophy of the KJ and soft tissues, regenerative tissue changes, hereditary diseases, trauma may be the reason of KJ diseases (Yakovenko, & Samoilenko, 2011).

Clinical symptoms of KJ can be: pain syndrome, soft tissue edema, deformation of the KJ, crackle when bending, local temperature increase, reduced mobility of the KJ. Despite the level of development of science in the world, medicine is not able to solve the main issue of orthopedics – the recovery of the affected articular cartilage. Basically, all the conservative measures are aimed at temporary pain relief only. When pain and functional disability cease to be resistant, the final method of treatment is total knee arthroplasty (TKA), which allows to stop pain syndrome in the shortest possible time, reproduce the limb axis and restore the lost function of the KJ (Buechel, 2006).

Total knee arthroplasty is considered to be a radical and effective method of treatment of osteoarthrosis of KJ. Despite the accumulated extensive experience of TKA, continuous improvement of endoprostheses, tools and techniques for their implantation, complications arise in 3-15% of patients at different times after the surgery. Among them are superficial and deep suppuration (0.2-9%), aseptic loosening of the components of the endoprosthesis (8-22.2%) (Epifanov, 2005). However, there are no clear rules for physical rehabilitation after TKA at present, that is why the issue of studying TKA is relevant.

The purpose of the study. To develop and practically substantiate an increase in the effectiveness of passive therapy after total knee arthroplasty in the long-term postoperative period of rehabilitation.

Material and methods of research

To accomplish the assigned tasks, the following research methods have been used:

- Sociological research method – questioning;
- Medical and biological research methods;
- Statistical methods.

To conduct a questionnaire to assess the patient's quality of life, the MOS SF-36 questionnaire – Health Questionnaire – 36 was used. This questionnaire has been developed in the USA and contains 8 scales and 36 questions. It allows you to assess the overall health of the patient. And indicates the level of physical and mental state, as well as social functioning.

Biomedical research methods of patients before and after rehabilitation treatment are carried out by using International tests. To characterize the pain syndrome and the functional state of the KJ, the International Knee Assessment Scale was used (Insall et al., 1976). The scale contains 6 points, where the normal indicator is 100 points, and in the presence of osteoarthrosis – 0 points. The quantitative assessment of pain experience is performed by the patient himself by using the visual analogue scale (VAS) (Huskisson, 1974).

The VAS is designed to determine the patient's subjective pain sensation at the time of the study. This scale can be used to determine the dynamics of pain intensity over 24 hours or 1 week. However, it should be borne in mind that memories of pain can be inaccurate and often distorted under the influence of other circumstances. VAS is a horizontal line 10 cm long with the inscription under the left edge "No pain", which means 0 points, and under the right - "Worst pain imaginable",

which means 100 points. The segment within the first 4 cm corresponds to the lack of pain, 5-44 mm – mild pain, 45-74 mm – moderate pain, 75-100 mm – severe pain. The patient should draw a vertical line across the indicated line in the place most appropriate to the intensity of pain at the time of examination. The advantage of VAS is its availability for statistical processing. The systematic measurement of pain by using the VAS gives a pattern of the dynamics of pain and the effectiveness of treatment.

Overall knee function was assessed by using the Oxford Knee Scale (Dawson, Fitzpatrick, Murray, & Carr, 1998). This scale consists of 12 points and allows the patient to characterize pain sensations, as well as to perform daily various activities. The dynamics of the functional state of the joints was assessed by using the WOMAC index (Osteoarthrosis Index of the University of Western Ontario and McMasters). The WOMAC scale includes 24 questions in 3 sections. The patient, answering the questions, chooses the answers that best describe his condition in points: from 0 to 100. An increase in the total number of points indicates a deterioration in the functional state of the KJ. To study the locomotor function of the KJ, the time (in seconds) was determined where the patient had to travel 15 m at the maximum pace.

For statistical analysis and modeling, a personal computer with an Intel Core2Quad processor with 4 GB of RAM in the standard configuration was used. To organize and form a matrix of data, tables and graphs, MS Office 2010 was used. The probability of data was determined by using the method of mathematical statistics by the Student's t-criterion.

The study was carried out from November 2019 to March 2021 on the basis of the rehabilitation treatment department of the Medical Center of the Karpaty State Medical Center in the town of Truskavets. The involvement of patients in the experiment was carried out as they entered physical rehabilitation on the 5-10th day after discharge from the hospital. The group consisted of 18 patients who underwent TKA at the age of 35 to 65 years (48.28 ± 2.89) with a diagnosis of osteoarthrosis of the KJ. The main selection criteria for the patients who took part in the study were the following: the lack of general contraindications to physical rehabilitation and their voluntary consent. The patients were divided into two groups: the control group – 10 (50.20 ± 2.81) and the main group – 8 patients (45.88 ± 3.37).

The rehabilitation process of the control group was carried out according to the standard physical rehabilitation program adopted in the treatment center:

- physiotherapy procedures: acupuncture, electrophoresis, muscle electrical stimulation, balneotherapy;
- therapeutic massage;
- nutritional correction to reduce the excess body weight of patients;
- a set of therapeutic exercises according to the methodology used in the medical institution.

The duration of rehabilitation of patients in two groups was 21 days (Burianov, Omelchenko, & Mikhnevych, 2009).The main group included the use of passive movement therapy by using the Artromot Active-K apparatus for a long period of time in addition to the above-described exercises(Apparatus Artromot K-1). The device is based on the method of motor passive therapy (CPM – therapy (Continues Passive Motion)). This method allows you to perform passive movements with the diseased joint. Exercises performed by the patient on the device do not cause painful sensations. During a mechanotherapy session, the KJ performs rhythmic passive movements, where, as a result of the procedures performed, edema decreases, blood circulation improves and pain is eliminated. All patients at the beginning of rehabilitation and at the end of rehabilitation treatment conducted a questionnaire to assess the quality of life. The data obtained during the initial questionnaire survey and testing of both groups were compared to assess the effectiveness of the proposed method of KJ rehabilitation in the postoperative rehabilitation period.

Results of research and their discussion

As a result of the performed rehabilitation, one can see the dynamics of updating the indicators of the functional state of the operated limb of patients of the main and control groups. It should be noted that after the completion of the planned course of rehabilitation, patients of both

groups showed positive changes in almost all parameters assessing the functional state of the KJ: an increased mobility of the operated joint and muscle strength, return of the correct walking stereotype on flat and uneven terrain, on the stairs.

The quality of life was assessed according to the "MOS SF-36" - "Health questionnaires – 36" research method. The questionnaire determines the assessment of the quality of life in the preoperative period at the beginning of the rehabilitation course and one month after its completion of 8 patients who underwent a course of recovery physiotherapy according to the method of therapeutic physical training developed by us, as well as 10 patients in the control group. The data are presented in Table 1.

Table 1

The main parameters of the quality of life of patients after TKA before and after rehabilitation

Parameter name	Quality of life parameters before rehabilitation		Quality of life parameters after rehabilitation	
	main group	control group	main group	control group
Overall health	13.7±1.6	15.6±0.9	22.7±1.4*	18.4±1.9
Physical functioning	14.5±1.8	16.1±0.8	24.2±1.5*	20.4±1.6**
Role physical functioning	4.3 ±0.7	5.2 ±0.8	5.7±0.6*	4.6±0.9**
Pain scale	4.8±0.7	6.8±0.7	4.2±0.2*	6.2±0.4
Viability	14.7±0.3	16.1±0.8	21.6±0.6*	17.7±0.8**
Social functioning	6.8±0.5	5.5±0.5	2.7±0.2*	4.8±0.5**
Role emotional functioning	5.1±0.3	3.6±0.1	5.6±0.2	4.2±0.3**
Mental health	15.6±0.7	14.9±0.8	25.7±0.5*	18.2±1.1**

Note * - significant differences in indicators before and after rehabilitation within the group ($p < 0.05$);
** - significant differences in indicators in the main and control groups after rehabilitation ($p < 0.05$).

According to the results of the table, before undergoing rehabilitation of the examined patients, all the parameters of both groups on the quality-of-life scales were reduced and amounted to: 4.8 ± 0.7 in the main group; 6.8 ± 0.7 in the control group before rehabilitation and 4.2 ± 0.2 and 6.2 ± 0.4 after rehabilitation according to the pain scale. It is obvious that pain syndrome, which is the main parameter in case of gonarthrosis and after arthroplasty, results in a decrease in physical functioning, the parameters of which were as follows: 14.5 ± 1.8 in the main group, 16.1 ± 0.8 in the control group before rehabilitation, and 24.2 ± 1.5 in the main group, 20.4 ± 1.6 in the control group after rehabilitation – according to the scale of physical functioning. A decrease in the indicator of the parameter of physical functioning is associated with the limitation of daily and professional activities depending on the handicap, namely the deterioration in the quality of life according to the scale of role physical functioning, the indicators of which were: 4.3 ± 0.7 in the main group, 5.2 ± 0, 8 in the control group before rehabilitation and 5.7 ± 0.6 in the main group, 4.6 ± 0.9 in the control group after rehabilitation; social factor of functioning, the parameters of which, according to the scale, corresponded to the following indicators: 6.8 ± 0.5 in the main group, 5.5 ± 0.5 in the control group before rehabilitation and 2.7 ± 0.2 in the main group, 4.8 ± 0.5 in the control group after rehabilitation.

Decrease in indicators on the scales of role emotional functioning (5.1 ± 0.3 in the main group, 3.6 ± 0.1 in the control group before rehabilitation and 5.6 ± 0.2 in the main group, 4.2 ± 0.3 in the control group after rehabilitation and viability (14.7 ± 0.3 in the main group, 16.1 ± 0.8 in the control group before rehabilitation and 21.6 ± 0.6 in the main group, 17.7 ± 0.8 in the control group after rehabilitation is the result of negative emotions and anxiety, which is clearly illustrated on the scale of

the mental state and is expressed by a decrease in its indicators: 15.6 ± 0.7 in the main group, 14.9 ± 0.8 in the control group before rehabilitation and 25.7 ± 0.5 in the main group (18.2 ± 1.1 in the control group after rehabilitation) As a result of changes in mental and physical health, a decrease in the parameter of general health occurs (13.7 ± 1.6 in the main group, 15, 6 ± 0.9 in the control group before rehabilitation and 22.7 ± 1.4 in the main group, 18.9 ± 1.9 in the control group after rehabilitation).

Changes in the parameters of the quality of life after undergoing rehabilitation of patients of the main group indicates a significant positive effect of the program of rehabilitation measures on the main components of the quality of life, namely, on the factor that has been eliminated as a result of arthroplasty and due to physical rehabilitation. In fact, this has resulted in an increase in the patient's self-esteem on the scales of physical and role functioning. According to the parameters of the scales of role emotional functioning, vitality and social functioning, an increase in indicators is obvious, explained by a surge of positive emotions as a consequence of the result obtained. But, the indicators on the scales of overall health, physical and role-based physical functioning are not high, since patients still cannot move and work on their own. At the same time, they objectively assess their state of health and possible prospects for the future, which is manifested in a low assessment of overall health.

In general, we can talk about the improvement of all the studied parameters of the quality of life after the completion of the physiotherapy program of patients of both the control and the main groups, especially when compared with the parameters of the quality of life after discharge from the hospital. Reliable results of improvement of the main group in comparison with the control group were obtained for all parameters of the quality of life. Consequently, the analysis of the quality of life of patients after TKA showed a decrease in the indicators of the quality of life in the pre-rehabilitation period and a significant recovery after the rehabilitation program in the late post-rehabilitation period.

The parameters for assessing the KJ were determined according to the International Knee Assessment Scale [11]. The dynamics of the study of knee arthroplasty patients showed that the parameters of the KJ assessment scale change depending on the rehabilitation in both groups, although to a different extent of significance and manifestation. The patients of the main group have a clearly pronounced tendency towards the improvement. The value of the KJ assessment scale increased by 33.4 points (from 42.38 ± 1.47 to 75.8 ± 6.9). The control group also differed in the improvements in indicators by 14.4 points (from 40.2 ± 1.39 to 54.6 ± 1.57) (Table 2).

Table 2

Dynamics of indicators of the KJ scale (points) after total arthroplasty

Groups	Before rehabilitation	After rehabilitation
Main	42.38± 1.47	75.8 ± 6.9*
control	40.2 ±1.39	54.6 ±1.57* **

Note * - significant differences in indicators before and after rehabilitation within the group ($p < 0.05$);
** - significant differences in indicators in the main and control groups after rehabilitation ($p < 0.05$).

A combined visual analogue pain scale (VAS) was used to assess the effectiveness of the proposed physiotherapy procedures and quantitatively assess the pain syndrome of patients of both groups before and after the course of physiotherapy. The dynamics of the study of total knee arthroplasty patients showed that the parameters of the pain scale change as a result of physiotherapy in both the main and control groups, but with different indicators of significance and manifestation. Under the influence of a specially developed program of physical exercises of therapeutic physical training, a statistically significant positive result of a decrease in the pain factor in the main group by 2.39 points (5.71 ± 0.11 to 3.32 ± 0.08) was obtained; the control group also

revealed an improvement: the parameters of the pain scale decreased by 3.78 points (6.16 ± 0.07 to 3.94 ± 0.06) (Table 3).

Table 3

Dynamics of VAS parameters

VAS scale parameters		Before rehabilitation (points)	After rehabilitation (points)
groups	Main	5.75 ± 0.52	3.38 ± 0.35*
	control	6.20 ± 0.34	3.90 ± 0.40*

Note * - significant differences in indicators before and after rehabilitation within the group ($p < 0.05$).

The general functional activity of the KJ after the total arthroplasty was assessed by using the Oxford scale. To determine the effectiveness of the proposed therapeutic physical training complex with the predominant influence of the use of physical exercises of patients of both groups, before and after a course of physiotherapy, the general function of the KJ was determined by using the Oxford scale for the KJ (Dawson, Fitzpatrick, Murray, & Carr, 1998). The dynamics of examination of patients after total knee arthroplasty showed that the parameters of the Oxford scale change as a result of physiotherapy in both the main and control groups, but with different indicators (Table 4).

Table 4

Dynamics of the Oxford Scale parameters after total knee arthroplasty

Indicators of the Oxford Knee Scale		Before rehabilitation (points)	After rehabilitation (points)
Groups	Main	34.13 ± 1.71	42.38 ± 1.47*
	control	30.40 ± 2.1	34.4 ± 1.54 **

Note * - significant differences in indicators before and after rehabilitation within the group ($p < 0.05$);
** - significant differences in indicators in the main and control groups after rehabilitation ($p < 0.05$).

Statistically accurate values of the parameter's improvement were observed after the completion of the rehabilitation course in the main group. The indicators of the Oxford scale changed of patients of this group by 8.2 points (from 34.13 ± 1.71 to 42.38 ± 1.47). The control group also showed an improvement by 4 points (from 30.40 ± 2.1 to 34.4 ± 1.54).

Dynamic indicators of the functional state of the KJ were assessed by using the WOMAC index. To analyze the dynamics of the main functions after total knee arthroplasty, it is shown in Table 5, which shows the total normalized value of the / WOMAC index in the study and control groups of patients. Significant positive dynamics of the patients of the main group under the influence of a special therapeutic physical training program was observed. The value of the index / WOMAC decreased in this group on the pain scale by 49%; on the morning stiffness scale by 59%; on the scale of joint physical function by 31% and the normalized value of the index by 37%. The indicators of patients of the control group under the influence of the standard complex of therapeutic physical training were lower and amounted to: 38%, 44%, 21%, 27%, respectively (Table 5).

To determine the locomotor function of the KJ after total arthroplasty, the time required for patients to travel 15 m at the maximum pace before and after physiotherapy was researched. Examination of patients showed that the test indicators change during treatment in both groups, but if in the main group we see a statistically important improvement after treatment using the developed therapeutic physical training program – a decrease in the travel time by 9.8 s (from 30.7 ± 1.7 to 20.9 ± 1.2), then the speed of movement of patients in the control group changed less: the travel time decreased by 5.1 s (from 29.4 ± 0.9 to 24.3 ± 0.8).

Table 5

Dynamics of the index / WOMAC indicators under the influence of rehabilitation

Functional Index / WOMAC	Groups					
	main			control		
	Before rehabili-tation	After rehabili-tation	% Dec-rease	Before rehabi-litation	After rehabili-tation	% Dec-rease
Pain scale (points)	421.2± 11.5	213.13± 21.2*	49%	445.9± 17.7	279.9 ± 29.5**	38%
Morning stiffness scale (points)	144.2± 14.3	59.6± 9.4*	59%	134.2± 8.1	75.8± 6.9	44%
Joint physical function scale (points)	1243.8± 22.2	867.8± 23.4*	31%	1123.4± 24.9	895.4± 26.2	21%
Normalized WOMAC index value (points)	1809.4± 29.1	1142.6± 30.3*	37%	1705.6± 33.6	1251.7± 36.4**	27%

Note * - significant differences in indicators before and after rehabilitation within the group ($p < 0.05$);
** - significant differences in indicators in the main and control groups after rehabilitation ($p < 0.05$).

Conclusions

As a result of the rehabilitation, the dynamics of recovery of indicators of the functional state of the operated limb is observed of the patients of main and control groups. It should be noted that after the completion of the planned course of rehabilitation, patients of both groups showed positive changes in almost all the parameters assessing the functional state of the knee joint: an improved mobility of the operated joint and muscle strength, the return of the correct walking stereotype on flat and uneven terrain, on the stairs, but with different extent of manifestation.

Under the influence of a specially developed program of therapeutic physical training, a statistically accurate positive result of a decrease in the pain factor in the main group by 2.39 points (5.71 ± 0.11 to 3.32 ± 0.08) was obtained; an improvement was also observed in the control group: the parameters of the pain scale decreased by 3.78 points (6.16 ± 0.07 to 3.94 ± 0.06).

Statistically accurate values of the parameter's improvement were observed after the completion of the rehabilitation course in the main group. The indicators of the Oxford scale changed of patients of this group by 8.2 points (from 34.13 ± 1.71 to 42.38 ± 1.47). The control group also showed an improvement by 4 points (from 30.40 ± 2.1 to 34.4 ± 1.54).

The dynamics of the study of knee arthroplasty patients showed that the parameters of the knee joint assessment scale change depending on the rehabilitation in both groups, but with different extent of significance and manifestation. The patients of the main group showed a clear pronounced tendency towards the improvement. The value of the knee joint assessment scale increased by 33.4 points (from 42.38 ± 1.47 to 75.8 ± 6.9). The control group also differed in the improvement in the indicators by 14.4 points from 40.2 ± 1.39 to 54.6 ± 1.57.

To determine the locomotor function of the knee joint after total arthroplasty, the time required for patients to travel 15 m at the maximum pace before and after physiotherapy was studied. Examination of patients showed that the test indicators change during treatment in both groups, but if in the main group we see a statistically important improvement after rehabilitation by using the developed therapeutic physical training program – a decrease in the travel time by 9.8 s (from 30, 7 ± 1.7 to 20.9 ± 1.2), then the speed of movement of patients in the control group changed less: the travel time decreased by 5.1 s (from 29.4 ± 0.9 to 24.3 ± 0.8).

The results of the overall assessment of the effectiveness of rehabilitation of patients showed that of patients of the main group, under the influence of a special program of long-term therapy and the apparatus method, a significant positive dynamics was observed. The value of the index indicators on the WOMAC scale decreased in this group on the pain scale by 49%; on the morning

stiffness scale by 59%; on the scale of joint physical function by 31% and the normalized value of the index by 37%. The indicators of patients of the control group under the influence of the standard complex of rehabilitation were lower and amounted to: 38%, 44%, 21%, 27%, respectively.

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SECTION II

NANOBIOSENSORS

Chapter 6. NUCLEAR BIOTECHNOLOGY

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Abstract. *Various new scientific and technological disciplines have emerged in the past century that largely shapes our modern world. Among those are quite a number of interdisciplinary fields such as nuclear electronics, radiomedicine, bioelectronics, etc. Though there exist meanwhile also various overlaps between nuclear technology and biotechnology, no such corresponding interdisciplinary field has yet been, however, officially acknowledged. Therefore we want to define in this work: "Nuclear Biotechnology" as a new field that covers a) the application of energetic irradiation techniques to biotechnology for precise destruction of unwanted cells, b) ion beam analysis techniques for the determination of elements or biomolecules in biological or biotechnological samples, c) biosensors produced by nuclear techniques for the detection of biomolecules, viruses and cells, d) nuclear track-based bio-actuators, e) nuclear track-based simulation of biological systems, f) biomimicking systems, and g) the application of methods from Nuclear Electronics for biotechnology. Apart from giving a general overview of this new discipline, we shall present some recent examples. They concern especially the various strategies for the production of biosensors based on nuclear tracks and the recent use of nuclear analysis techniques for their optimization. We also give an outlook on their further development.*

Keywords*: nuclear tracks, biosensors, nuclear techniques.*

Introduction

The past century was a time of revolutionary development of many important new scientific and technological fields that hitherto dominate our lives. This holds on the one hand for the enormous progress that organic chemistry made when it became possible to reveal the detailed structures of even large protein molecules and thus also of their precise functionalities. This led to the emergence of molecular biology and thus to a fundamental understanding of the processes going on within and between cells. Simultaneously modern genetics emerged that put the elemental biological processes onto a solid scientific basis, thus depriving fatal ideologies from their seeming justification. It also holds for the dramatic post-war emergence of electronics with modern data processing as its prominent spin-off. A few decades later, mechanical engineering found a renaissance by turning to micro-and nanoscale dimensions and towards robotrics. Also these disciplines had some impact upon the rapidly changing fields of biology and medicine, which among others led to the new areas of biotechnology and medical technologies with their rapidly growing social and economical importance.

Last not least, one should mention the simultaneous emergence of nuclear and radiation sciences and technologies in this context. Already in the early stages, biological effects of radiation became known that led on the one hand to the disciplines of dosimetry and radioprotection, and on the other hand to radiomedicine, especially in the context of fight against cancer. Apart from these areas of major interdisciplinary activities, nuclear and radiation skills and technologies were applied to biology and/or medicine for e.g., human diagnostics with X rays, nuclear magnetic resonsonance (NMR) and positron emission tomography (PET), medical filtration with nuclear track filter foils, food irradiation, and nuclear analysis techniques such as proton induced X-ray emission (PIXE). Recently other challenging new possibilities emerged, such as cell surgery with ion nanobeams, nuclear track-based biosensing, or the construction of biomimetic systems such as artificial neural networks. Therefore we think it is time to unite all these diverse developments within a new frame, for intensifying the interdisciplinary cooperation in this new field. We denoted this new frame as "Nuclear Biotechnology", which we define here as that part of biotechnology that makes use of nuclear and radiation techniques and skills, and of nuclear spin-offs such as nuclear electronics. According to this tentative definition, Nuclear Biotechnology consists of –

a) the application of energetic irradiation techniques to biotechnology *(such as given for the sterilization of food or instruments, organic waste decomposition, bioenergy conversion techniques and the production of nuclear track filters for removal or separation of bacteria or viruses), and including also the recent advances of ion micro- and nanobeams for future cell surgery*,

b) ion beam technologies for elemental analysis of biological samples *(such as the Nuclear Analysis techniques: PIXE, Rutherford backscattering (RBS), elastic recoil detection (ERD), nuclear reaction analysis (NRA), neutron depth profiling (NDP)), secondary ion mass spectrometry (SIMS) and their advanced 3D tomographic expansions)*,

c) nuclear track-based biosensors for the detection of inorganic molecules required in biology *(such as NH_3, O_2, H_2O, etc.)*, of biomolecules *(such as enzymes, hormons, DNA, etc.)*, of viruses and of various types of cells *(such as bacteria, plant cells, animal cells and human cells)*,

d) nuclear track-based bio-actuators *(such as thermo- and pH-responsive valves)*,

e) nuclear track-based simulation of biological systems *(such as the use of etched ion tracks in thin polymer foils as simple non-biological models to understand the complex transport processes going on in cell membranes)* for future technological applications,

f) biomimetic systems *(using nuclear track foils as artificial neural networks, if arranged in a way that individual tracks can electronically interact with each other)*, and

g) the application of methods from Nuclear Electronics for biotechnology *(such as the use of coincidences and multiplexing)*. It is still a matter of discussion whether one should also include the fields of radiation applications for medical diagnostics and tumor therapy - or not, as they already form traditionally an essential part of radiomedicine.

In this paper we will first give a general overview about the required fundaments of Nuclear Biotechnology, and then illustrate this field for some specific problems.

1. Fundaments of Nuclear Biotechnology

The fundaments of Nuclear Biotechnology are manifold. On the one hand, there are the purely ***nuclear fundaments*** that deal with nuclear reactions applied to biotechnology via nuclear analysis techniques such as PIXE, NRA, NDP or RBS. On the other hand, the continuous energy transfer to the biological targets by the radiation along its trajectory is of importance, which leads to a gradual decrease in radiation intensity (for electromagnetic radiation) or in projectile energy (for energetic particle radiation, respectively) with pathlength, until these particles come to rest at their so-called "range". The energy transfer leads to both structural and chemical radiation damage especially in biomatter and other organic targets (such as polymers). In the case of radiation effects on humans, this is described by the disciplines of radiomedicine and dosimetry. Last not least, the radiochemical reaction products might suffer diffusional redistribution in the biological targets that can give rise to secondary biological reactions.

The ***biological fundaments*** refer to various objects of increasing complexity, from simple inorganic molecules such as (O_2, H_2O, CO_2, CH_4, NH_3, etc.) that form the fundamental basis of any metabolism, the biomolecules, ranging from simple ones (such as glucose, urea, cholesterol, etc.) via proteins (enzymes, hormons), DNA and RNA, towards the elemental DNA-based organisms such as viruses and cells (bacteria, plant and animal cells, human cells), and their agglomerations towards complex organisms such living individuals with their individual organs.

The ***chemical fundaments*** refer, on the one hand, to the steric structures of the above-mentioned biomolecules, and to their reactions with each other. Another important field is also electrochemistry with nanofluidics as its recent spin-off, as in the nanoscopic world of basic biological structures many components are charged so that Coulombic interactions largely determine their behavior. Good knowledge of nanofluidics is also important for constructing efficient nanometric biosensors.

Electronic and computational fundaments are necessary on the one hand to understand both the individual and collective neuronal functions. On the other hand, knowledge in electronics is required to develop the circuitries for data recording, amplification and processing of e.g., biosensors, and to integrate the newly emerging biosensors into the well-established scheme of contemporary semiconductor electronics (such as their description by four-pole parameters in network theory). Furthermore, special techniques that are well-established since long in Nuclear Electronics (such as the use of coincidences, multiplexing, pulse shape analysis, multichannel analysis, routing, etc.) but still rather unknown in biotechnology might yield advantageous pulses to the further development of that field.

As there exists numerous books and articles about all these fields, we refer to the available literature for further information.

2. Nuclear Biotechnology

2.1. Application of energetic irradiation techniques to biotechnology

Energetic electromagnetic (such as X or gamma rays) or particle (such as electrons, protons, or heavier ions) radiation may destroy tissue by the transfer of excessive energy, via either electronic target excitation (i.e. radical formation) or ionization or by nuclear collisions (leading to the breaking of existing chemical bonds). Thus biomolecules with vital functions may be denaturated or even destroyed, and some of the newly formed radiochemical reaction products will act as cell poisons. Exactly this is what radiative tumor therapy strives for: a maximum energy density shall be delivered within malign cells. However, benign cells in the vicinity should be affected as little as possible by the radiation, to avoid adverse secondary effects of the patients. In the case of deep-lying tumors, this task is best achieved by high-energy (~50 MeV to some GeV) light (such as protons) or medium-heavy (such as e.g., carbon) ions, which explains the contemporary great interest in the construction of such energetic ion accelerators in medical facilities [1, 2].

A new emerging field is the so-called “cell surgery” [3]. With this, the radiative destruction of organelles within individual cells has been denoted, to understand better the organelle's functionalities by comparison of the cell behaviour with and without the existence of these

organelles. To achieve this goal, ion beams of truely nanometric diameters have to be delivered on target areas of nanometric sizes within nanometric localization accuracy, which signifies the presently utmost challenge in ion beam technology. The path presently followed is to feed energetic ions into sub-micrometer glass capillaries, where they can be proliferated by the focusing [4] and guiding [5] mechanisms within the desired diameter towards the target.

Destruction of pathological viruses, bacteria, fungi or insect eggs on both medical instruments and in food is another important field of energetic irradiation, to avoid the proliferation of infectous diseases. Great care must be undertaken in food irradiation to select the optimum irradiation dose, on the one hand to efficiently destroy the adverse microorganisms or at least to impede their reproduction capability, but on the other hand to keep the radiative food denaturation at its lowest possible level. Meanwhile a lot of work has been invested to correlate the radiation damage in various types of food with the corresponding irradiation dose; among the most favored methods are thermoluminescence (TL), photostimulated luminescence (PSL), and electron spin resonance (ESR) [6, 7]. Especially the latter has gained great importance to probe the radiation-induced radical formation as a dosimetric representative of the irradiation dose, so that nowadays the delivered radiation doses can be controlled rather reliably for many types of food. This should prevent or at least hinder future abuse of excessive radiation. (Note, however Dodd's [8] earlier objection that “radicals per se are not harmful and many natural biological processes involve radical formation. Human life itself would be impossible without that ubiquitous radical molecular oxygen. The presence of long-lived radicals in irradiated foodstuffs, provides no cause for concern, because they appear either to be indistinguishable from naturally occuring radicals or are trapped in inedible parts of the food”). Radiation has also been proposed for future use in organic waste decomposition [9] and bioenergy conversion technology [10, 11], but to our knowledge this was not yet applied technologically.

Swift heavy ion beams have already been successfully used for the production of nuclear track filter foils since decades. These are usually thin polymer foils (typically ~10 μm thick polyethylene terephthalate (PET, mylar), polycarbonate (PC, makrofol) or polymimide (PI, kapton) foils), irradiated with heavy ions (with atomic masses ranging from Ar to U) at energies of some 100 MeV to a few GeV). The trails of radiation damage therein, the so-called “latent ion tracks”, can be easily etched to give rise to parallel nanopores with various lengths and shapes [12]. In the case of nanometric diameters, such nanopores even allow the “ultrafiltration” of virusses from liquids [13-15], which is usually not possible with conventional filters. Apart from this, these etched nuclear tracks can well be used in biosimulation and biomimetic experiments as well as substrates for novel biosensors, see below.

2.2. Application of nuclear analysis techniques to biotechnology

Nuclear analysis techniques (such as PIXE) can be helpful for biotechnology if the knowledge of trace elements in some biological sample or in some biotechnological device (such as a biosensor) is required. By making use of nuclear techniques such as RBS, ERD, SIMS, radionuclide detection analysis, NDP, etc., it is even possible to determine the corresponding depth distributions of these elements. The use of ion microbeams in such cases enables one to probe very narrow sample areas (in the order of a few μm^2 or less) in detail, or to determine the overall 3D elemental distribution in the sample by scanning the ion beam over a larger sample area.

The shapes of parallel nanopores used for biosensing (see below) can be controlled not only by the relatively expensive, difficult and time-consuming scanning or transmission electron microscopy (SEM, TEM) techniques, but also by the much easier ion transmission spectrometry (ITS) [16]. The ITS technique makes use of the energy loss spectrum of monoenergetic ions (preferentially α particles from a radioactive ^{241}Am source (E_α = 5.5 MeV) transmitted through a thin foil of any material. This spectrum sensitively depends on the depth distribution of the free volume – hence also of pores - in that foil which can be converted to the average pore shape.

Last not least one should not forget to mention the X ray and neutron small angle scattering (SAXS, SANS) techniques which yield detailed complementary three-dimensional structures of larger nanoparticles, and which therefore have been applied with great success since a few decades

to decipher the precise molecular arrangements in organic macromolecules such as proteins and viruses. They should also be useful tools for the production control of artificially designed macromolecules.

Nowadays nuclear analysis techniques are usually combined with mathematical techniques for the sake of improved data processing (such as background subtraction, noise reduction or deconvolution techniques, and tomography) to obtain results that exceed the usual spatial or concentration resolution and simple one-dimensionality of the above basic techniques. Also, extensive computer simulations of e.g. the diffusional processes going on in biomaterials (such as glucose sensors [17]) have contributed much to our understanding of these systems that were useful for biosensor optimization.

Eventually, if a suitable element can be attached unambiguously to some given biomolecule, this element might then act as a marker for nuclear analysis for that molecule, so that its concentrations and distributions within some biological or biotechnical structure can be obtained that are else inaccessible. Let us give a few examples:

1) By means of PIXE, one can determine the trace element spectrum in any biological or man-made sample. As these spectra give unambiguous fingerprints for each sample, this technique is widely used since long in e.g., forensic science (e.g., to prove which victim was killed in some crime with which gun, etc.) [18-20], in ecology (e.g., to probe the origin of dust particles in a given ambient [21, 22]), or in archeology (e.g. to probe which flintstone from a given ancient Polynesian site was taken from which volcanic island in the Pacific, to derive in this way the ancient overseas trade routes [23], or to probe the data scattering of trace elements in pottery found in ancient Near-East settlements, to get in this way an information about the economic importance of these sites in earlier times even if corresponding written records are missing [24].

2) A similar question concerned the purity of nuclear track-based biosensors, as harmful or even poisonous trace elements (such as Al, Hg, Cd, As, etc.) in larger quantities could prevent the possible future in-vivo incorporation of such biosensors. For this sake, we performed a series of examinations on track-based glucose sensors. To obtain a complete information and to characterize the sensor production steps better for their optimization, we examined not only the final biosensors themselves, but also the components for their production. This concerns the polymeric substrates (PET and PC), the carrier liquids {double deionized water (DDW), different phosphate buffers (PB, PBS and PB-Triton, prepared with sodium dihydrogen phosphate and di-sodium hydrogen phosphate dodecahydrate) as well as 2-(N-Morpholino)ethanesulfonic acid hydrate (MES hydrate) buffers}, the required enzyme linkers {N-ethyl-N_-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC) and Hydroxy-2,5-dioxopyrrolidine-3-sulfonicacid sodium salt (sulfo-NHS)}, the enzymes {glucose oxidase from *Aspergillus niger* (GOx) and cholesterol oxidase (CHOx)} in MES buffer solution and finally the analyte solutions (D(+)Glucose anhydrous and cholesterol) in MES buffer.

Fig. 1 gives a representative PIXE spectrum, and Table 1 compiles all results qualitatively. To obtain the trace element abundance in EDC, S-NHS, GOx and CHOx alone, the spectra of the corresponding carrier liquids were subtracted from the measurements. Then one finds that the base materials contain only few pronounced trace elements (foils: Ca; solutions: Ca, Na, Cl); the additional finding of strong phosphor abundance in phosphate buffers is, of course, trivial. Practically all used bioanalytical materials show an amazing purity after subtraction of the trace element spectra of the pure liquids from the solutions; the only exception is cholesterol which has a major abundance of P. Less abundant tracers are: Ti, Al, Si in the foils; Fe, Ti, S in DDW; S, K, Br in buffer solutions and none in the enzyme linkers and in the enzyme GOx itself.

The final glucose sensors contain only P as major trace element which, according to our overview, must stem from the previous use of the phosphate buffers; apparently even 5 times washing of the final sensors with DDW is not yet sufficient to remove all phosphate adsorbants. The only two minor trace elements (with concentrations less than a percent of the P concentration) are Fe and Zn, so that one can conclude that the prepared sensors are indeed very pure devices and do not contain any biologically doubtful components.

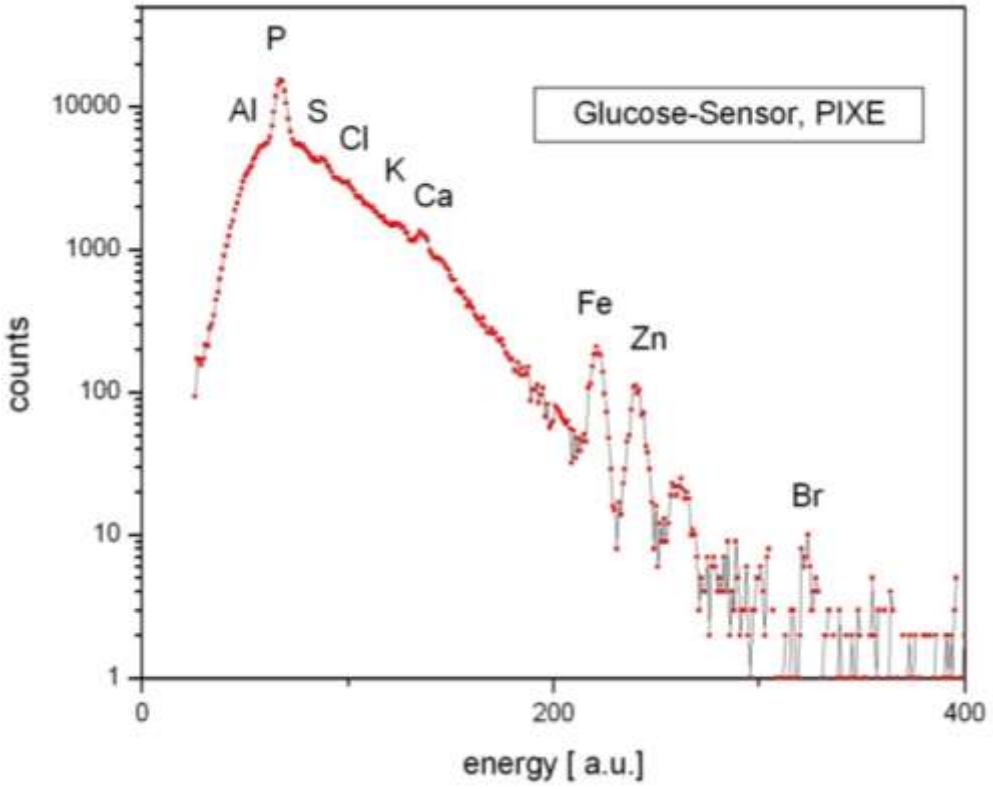

Fig. 1. A typical PIXE spectrum, as shown here for the final prepared glucose sensor. The individual elemental peaks are superposed as usual to a large background and therefore have to be subtracted for proper data evaluation. Note the logarithmic Gamma count scale which signifies that even pronounced structures at high energies signify in reality very low abundances

Table 1

Qualitative comparison of the trace elements in all components required for the production of glucose sensors (though CHOx and cholesterol were already characterized additionally in this connection, cholesterol sensors have unfortunately not yet been prepared). X highly abundant, + moderate abundance (i.e. less than ~10% of X), - small availability (i.e. less than ~1% of X), blank: not detectable

Type	Material	PIXE	Ca	Cl	Fe	P	Zn	Na	K	Ti	S	Br	Al	Au
Substrate Foils	Polycarbonate	5765	**X**											
	PET	5775	**X**		-					-				
Carrier Liquids	D. Dist.Water	5775	**X**	**X**	-					-	-			
	MES	5776	-	**X**	-		+		-		-	-		
	Buffer PB	5777	-	-		**X**		**X**						
	Buffer PBS	5785	-	**X**		**x**		**X**	+				-	
	Buf. PB-Triton	5780	+	**X**	+	**X**	-	**X**	+	-	-		+	+
Enzyme Linker Solutions	EDC in MES	5778		**X**							+	-		
	S-NHS in MES	5782	-	**X**							-		-	-
Glucose sensing	GOx in MES	5784	-	**X**							-		-	-
	Glucose	5781												
	Gluc. Biosensor	5768	-	-	+	**X**	+				-	-		
Cholest. sensing	CHOx in MES	5783	-	**X**	-				-		+		-	-
	Cholesterol	5779	**X**	-	**X**	**X**	**X**	+					-	

3) During the development of the above-mentioned glucose sensors, also the question arose whether in a given sample the enzymes really had fully clad the inner surfaces of the etched tracks, or whether certain track regions might not have been clad satisfyingly. In the case of enzymes that contain heavy metal ions (such as laccase that contains Cu ions), the question could have been solved by measuring the Cu depth profiles along the etched tracks by applying RBS or NRA. However, in the case of GOx distributions in which we were interested, this approach fails as GOx does not contain any of such heavy elements. Therefore we first attached boric acid to the enzyme GOx (that had been bond to the etched tracks before), and thereafter considered the adsorbed boron isotope 10B (the abundance of which is about ~20% in boron) as a marker for the GOx distribution. The depth distribution of this isotope was determined by the neutron depth profiling (NDP) technique [25] via the nuclear reaction $^{10}B(n,\alpha)^{7}Li$ with neutrons at the Prague research reactor. NDP enables probing depths up to several μm at ppm concentrations and is therefore ideally suited so that we could probe the total boron distribution well [26]. The results indicated that the enzyme clad indeed the etched track surfaces in greater depths completely, whereas some depletion was observed within the first surface-near 200 nm. This might be attributed to incomplete attachment of either GOx to the track openings, or of BO_3^{3-} to GOx which enabled the marker removal during the subsequent washing of the sample. Furthermore the result reconfirmed that the tracks had conical shapes (due to previous track etching from one side only) and that the etched cones had lengths of only 1.5 μm, i.e. that track etching was stopped in this case long before complete etchant breakthrough was accomplished, so that the track shapes were indeed funnel-like.

2.3. Nuclear track-based biosensors

General overview. Since a few decades, the field of biosensors is greatly expanding. For measuring and quantifying biological signals, the latter ones have been transformed to nearly every possible physical or chemical parameter in partly quite sophisticated ways. Bio-induced weight changes have been determined by ultrasensitve scales; bio-induced changes in optical parameters such as frequency, optical absorption, scattering, or polarization have been exploited; changes in pH values are exploited, and the determination of changes in electric parameters such as conductivity and capacity are commonplace, to mention just a few examples. Also electrochemical approaches are widely spread, such as realized by e.g., the so-called Clarc cells where the determination of oxygen consumption by a characteristic potential drop at a working electrode is used to indicate e.g., the existence of some biomolecule or some enzymatic cell activity [27], or even the presence of living cells.

Biosensors with micro-or nanochannels: In tasks which are devoted to the construction of biosensors, micro-or nano-channels are useful. On the one hand, the large inner surface of porous materials as compared with their external surface enables one to deposit there a large amount of reacting agents (such as enzymes), thus enhancing the sensor's response intensity greatly. On the other hand, the tiny volume of individual channels enables one to work with smallest quantities of bio-liquids (less than a femto-liter per etched track) which makes cheap and sensitive analysis possible. Furthermore, there is hardly any risk of mixing liquids from neighboring channels, and even mixing of two liquids in the same nano-channel imposes great difficulties as at nano-channel dimensions (corresponding to Reynolds's numbers below 2000) laminar flow dominates, whereas turbulent flow is required for mixing.

Two basic geometries exist. Nowadays, the channels are most frequently embedded as narrow linear depressions within planar surfaces of, e.g. polymers or ceramic materials, into which bioliquids are embedded by capillaric forces. Thus complex two-dimensional micro- or nanofluidic circuits with pumps, sensors, actuators, etc. are be realized that resemble in their outer appearance somewhat the conventional electronic circuits. The most impressive examples of this concept are the so-called labs-on-chip designs that are presently under development nearly everywhere in the world, and that should enable in their final stages highly complex, though rapid and cheap analysis processes of a whole plethoria of biologic parameters. Though nowadays often the capillaric channels are produced by non-nuclear techniques such as molding and printing, nevertheless also

nuclear-based techniques such as ion beam sputtering through masks or microbeam or focused ion beam (FIB) sputtering still play an important role, specifically for research and development.

Another geometric possibility is to arrange the micro-or nanochannels perpendicularly to the substrate surface. This is accomplished by using e.g., polymer or ceramic foils (either self-carrying or attached to a substrate) with perpendicular parallel pores. These nanopores can be e.g., those ones that emerge in thin porous Al_2O_3 sheets as a result of specific electrochemical deposition and/or dissolution processes on Al electrodes in selected electrolytes [28]. However, such ceramic membranes have disadvantages insofar they are highly brittle and also readily dissolve in non-neutral solutions.

For contrast, polymer films or foils that were irradiated by swift heavy ion beams and subsequently etched (thus creating so-called “etched ion tracks”) are flexible, largely resistant against acidic attack and can be produced with any desired thickness from a few nm up to a few μm (if on a substrate), or from a few μm up to ~100 μm or so (if self-carrying). One also can prepare foils with arbitrary pore-containing and pore-free regions (by ion irradiation and/or etching through masks). The pore density can be tailored to any value (by adequate choice of the fluence of the track-forming ions) and the selected etching technique enables one to obtain any desired pore shape (conical, cylindrical, funnel-type, barrel-type etc.) and diameters between a few nm and some μm. Further, embedding of the bioliquids within micro-or nanopores enables one to largely prevent their rapid drying (due to the small pore openings, as compared with long micro-or nano-channel systems within planar surfaces), which is an inherent danger in the above-mentioned planar micro-or nanofluidic devices. Instead of forming ion tracks in polymers by irradiation with energetic ions and subsequent etching, one can also use micro- or nano-beams to drill micro- or nano-pores or narrow surface channels for biosensors.

In the light of these advantages of porous substrates it is obvious that sensors of the Clarc type have been combined with nanoporous foils already rather early [29]. Here, the large (as compared with the foil's geometrical surface) overall surfaces of parallel etched tracks within swift heavy ion-irradiated polymer foils were covered with thin layers of gold, that acted as the Clarc working electrode and that was opposed to a Ag/AgCl reference electrode. In this way, the oxygen content within e.g., aquaria or swimming pools could be determined permanently via reading of the Au potential. Apart from this potentiometric approach (which can be transferred to an amperometric one by forcing the potential to be constant via adding a sensing current), also other concepts have been developed to make use of such nanoporous foils for biosensing. Their principle is usually to clad some reagent (e.g. an enzyme) onto a (polymeric or TEMPOS-type (i.e., an irradiated and etched SiO_2 layer on a Si substrate [30]) substrate on either its surface and/or on the etched track walls. Table 2 summarizes in chronological sequence the different sensing concepts that have been developed in the past decades. All have in common that the presence of some analyte is detected by some change in the electrical signal that emerges upon the reaction of the analyte with the reagent.

Apart from the above-mentioned track-based Clarc sensor for O_2 gas, also other track sensors for the detection of inorganic gas molecules in biological metabolism have been designed, e.g. for the sensing of water vapor [31] or of ammonia [32]. Meanwhile there exist also track-based sensors [33-35] for determining a number of biomolecules (such as streptavidin, immunoglobulin, antibodies to ricin [36], glucose [37], urea [38], peroxides [39], vanilline, cafeic acid, ferulic acid, indigo carmine, ABTS, etc. [40, 41]) and viral sequences (such as those of H1N1, i.e. the Mexican swine flue [42]). These sensor concepts with individual foils can be expanded to systems with sensor foil cascades; first tests have shown that this leads to improved detection sensitivities and signal heights [43]. Also the insertion of central membranes into sensors with etched tracks [44] enables one to obtain higher detection sensitivities [45], and additionally such sensors act as frequency passes so that the sensors become rather insensitive against ambiental low- and high-frequency electrosmog [46].

Another interesting tendency shows up, since it has recently become possible to transform etched ion tracks into transistor-like structures, by changing the usually only negative surface charges on the ion track walls into npn or pnp sequences [47, 48]. In a next step, by additionally

enzyme-functionalizing these structures, it might become possible to expand them towards ion track biotransistor, in analogon to the already existing enzyme monolayer-functionalized field-effect transistors for biosensors applications [49].

Table 2

Overview about the different concepts for ion track-based biosensing

Concept, reference	**Description**	**Notes**
Charge transfer [47]	Some charges are extracted during chemical reaction of analyte with reagent towards conducting polymer substrate within tracks for subsequently registering	Conducting layer in between track surface and reagent necessary; hitherto poor sensor lifetime and charge extraction efficiency. Improvements planned by combining with TEMPOS structures [30]
Pore blocking [36, 39]	Narrow pore opening of a conically etched track is blocked by adsorption of an analyte molecule to a suitable receptor molecule on the track wall, hence transmitted current decreases	Single etched tracks required, hence very low currents. Very careful track preparation necessary. But detection sensitivity goes down to individual molecules
Polymeric electrolyte [32]	TEMPOS structures applied, polymeric electrolyte within etched tracks on Si changes conductivity upon gas adsorption; current enhancement by embedded nanoparticles.	Feasibility of concept verified for ammonia sensing. Application to other bio-relevant gases possible.
Clarc sensor [29]	Potential on Au tubule-type electrodes changes with dissolved oxygen concentration in neighboring electrolyte; calibration by opposed Ag/AgCl reference electrode	By forcing the potential to be constant via adding a sensing current, this potentiometric concept can be transferred to an amperometric sensor
Product enrichment [37, 38, 41]	By accumulation of charged products of analyte-reagent reactions in the confinement of narrow tracks, track conductivity increases	Analyte and reaction product solutions must have different conductivities; etched tracks must have large aspect ratios. Concentration intervals cover 5-10 orders of magnitude, minimum concentrations may range down to some pM
Latent track blocking [43]	Adsorption of analyte molecules to reagent molecules bond to irradiated foil surface blocks track entrance partly, thus spike amplitudes decrease with increasing analyte	Thin polymer foils with latent tracks yield current spike emission (instead of constant currents through etched tracks)
Gating of transmitted current [42]	Change of charge state of DNA bond to narrow pore opening of conically etched tracks upon reaction with viral sequence leads to different gating signals; transmitted currents increase with increasing analyte concentrations.	Synthetic DNA strands required; universal detection of any viral sequence possible. For H1N1 detection: nM sensitivity
Sensors with central membranes [45, 46]	Membranes lead to higher product enrichment by hindered product diffusion; polarization effects across the membranes lead additionally to higher detection sensitivity	Membranes act as frequency filters in the 5-50 kHz range; two different analytes can be studied on two different sides of one foil only

In contrast to ion-track-based biosensors, the use of ion micro-or nanobeams for biosensor production is still in its infant stage. First examinations were devoted to derive the changes of the polymer foil's electronic properties by high dose proton microbeam irradiation [50], and concepts have been developed to design cell sensors by combining large microbeam-created deep depressions with etched ion tracks in thin polymer foils [51], Fig. 2. The deep depressions are expected to be favorable for cells to accomodate therein, and they also shall serve for reducing the cell's potential energy so that the biomolecules emitted from them (i.e., metabolic products, signal molecules for cell communication and, in case of bacteria, also quorum sensing molecules) will rather remain within them, than escape to the ambient. The latter molecules that act as fingerprints

for the given cell types shall then be extracted to the opposite foil side via some pressure or electrical potential gradients, where they can be analyzed in a clean ambient.

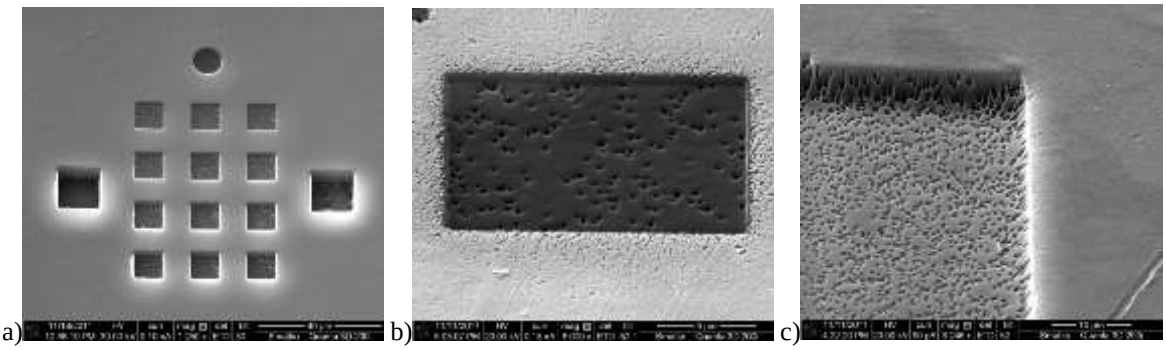

Fig. 2. First test structures that might serve as substrates for future cell sensors. The10 µm x 10 µm x 0.5 µm large depressions in the center of a) and in b) and c) were produced by focused ion beams, and the narrow circular depressions on the bottom of the large depressions are etched ion tracks. To restrict ion track etching to the microbeam depressions, the foil surface was coated with a thin non-wetting teflon-like coating. a) Test measurement with several depressions of various shapes and dimensions, overview; b, c) details; a, b) used foils with low track density – 1×10^{6} cm^{-2} and c) high track density – 1×10^{9} cm^{-2} ion track densities; b) sample observed under tilted angles

2.4. Nuclear track-based bio-actuators

There has been done a tremendous effort in the development of responsive polymers which can exhibit reversible or irreversible changes in physical properties and/or chemical structures to an external stimulus such as pH, temperature, ionic strength, light irradiation, mechanical forces, electric and magnetic fields, specific analytes, external additives (ions, bioactive molecules, etc.), or a combination of them [52]. However, to our knowledge the production of track-based bio-actuators that are responsive to the above-mentioned parameters has hitherto hardly been reported, apart from nuclear track valves clad with thermo- and pH-responsive gels [53].

2.5. Nuclear track-based modelling of biological systems

Etched ion tracks in thin polymer foils exhibit many of the nanofluidic properties that also exist in cell membrane channels. Therefore intense work has been invested in the past decade to use etched ion tracks as simple models for the more sophisticated biological structures, see e.g., Refs. [53, 54]. In this way, examinations could be performed to probe theoretical models on complex transport processes even far beyond the restrictions that natural membranes give, as regarding the pH values, the track lengths, diameters and shapes. The latest characteristic example of that kind was the Anomalous Mole Fraction Effect (AMFE), where the conductance through a pore is lower in a mixture of salts, than in the pure salts of either species at the same concentration [54]. Once a thorough understanding of this natural processes had been obtained by means of ion track studies, these new insights rendered the earlier explanation of this effect (that ions in narrow pores would migrate within single files) obsolete and indicated that this effect reflects the preferential selectivity of the pore for one cation species over the other (as formulated theoretically by [33]). This improved understanding may be helpful to design improved new biotechnological concepts, e.g. in desalination plants.

2.6. Biomimicking

As one has realized since long that biological systems are usually superior to man-made technological ones due to their much higher degree of inherent intelligence and sophistication [55], attempts to understand and copy the biological design principles have been undertaken since long. Meanwhile the compilation of such *biomimetic approaches* fills whole books or book chapters [56, 57]. Therefore it is obvious to search also for the possibility to overtake biological principles in

nuclear track-based fields. This is indeed possible. Let us look for example at the way how pores in cell membranes work. One can overtake at least part of the corresponding sophisticated construction principles to create synthetic nanopores with similar functionalities [35, 58-62].

An example for bio-mimicking is to simulate the emission of electrical current spikes from neurons by current spikes emitted from latent ion tracks upon application of a sufficiently high voltage [63], and letting then many of these spikes interact with each other, thus building up simple artificial planar neuron-type networks [64] after the example of the human brain. Similarly, the biological information that is obtained from some track-based biosensor can also be transmitted in pulsed form as this is usually the case in living organisms [65].

Let us look at logic decision-making that is commonplace for all creatures possessing neural networks. In [62] it was shown that functionalities such as logic AND or OR decisions can also readily be realized in track-based TEMPOS structures [60] which may be understood as simple planar artificial neural networks, the ion tracks overtaking here the role of neurons. Though in this example the driving signals to trigger decision-making processes in the TEMPOS structures were just electrical potentials applied to some external electrodes, it is only a short additional construction step to transfer biological signals into electrical potentials that can then be fed into the logic TEMPOS system, to arrive at future decision-making biosensors.

It was historic commonplace among Namibian natives that oistrich egg shells had a disinfecting action so that drinking water could be stored therein for a long time in hidden places in the Namib desert, without the danger of fouling. Mimicking the nanoporous structure of these egg shells that allows breathing but prevents germs to enter, microporous etched ion track foils were clad with TiO_2 nanoparticles and thereafter also showed a sterilizing effect [66]. Whereas tomatoes stored in normal closed glass vessels at ambient temperature (~20°C) were spoilt after only two days, they could be kept relatively fresh still after about 10 days in vessels that were hermetically covered with such foils.

2.7. Application of methods from nuclear electronics for biotechnology

It is not only possible to overtake biological strategies for nuclear track-based technologies, but vice versa it also appears possible to overtake strategies developed for nuclear science – as found e.g., in nuclear electronics – to biotechnology, specifically to biosensing. This might especially hold for coincidence (and/or anti-coincidence) techniques which are nowadays commonplace in all kinds of nuclear activities, and which might still yield dramatic improvements in biotechnology. In this case, two signals measured independently from each other but describing the same biological effect will have to be compared with each other (e.g., the charges extracted from enzymes reacting with their analytes and the concentrations of the corresponding enzymatic reaction products of that analyte). Signals that show up coincidently with both techniques will then be accepted, whereas others will be rejected. In this way, it should be possible to improve the detection efficiency of biosensors still by several orders of magnitude. Another electronic strategy that seems recommendable to be overtaken by biotechnology is multiplexing.

Summary

We think that meanwhile the amount of material having been accumulated by the overlap of nuclear technology and biotechnology has reached such a large amount that it justifies the introduction of a new interdisciplinary field of technology, which we denoted as "Nuclear Biotechnology". A tentative list of topics of Nuclear Biotechnology was given, and some yet unpublished examples of this discipline were presented – especially in the field of track-based biosensing.

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Chapter 7. NANOBIOSENSORS WITH NUCLEAR TRACKS AND EMBEDDED MEMBRANES

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Abstract. *Various biosensor strategies were developed in the past two decades that make use of etched nuclear tracks in thin polymeric foils. One of them uses the enrichment of charged enzymatic reaction products in the confinement of very narrow tracks, for enhancing the detection sensitivity. Recently the attempt was undertaken to separate these etched tracks into two compartments by membranes to determine two different biomolecules simultaneously. At that occasion it has turned out that, by inserting the same enzyme into both compartments, the sensing accuracy could be enhanced greatly, as compared with simple etched tracks only. Further, such sensors act as frequency filters which is useful for sensor operation in ambients with much electrosmog. In this paper, the way to produce such membrane-containing sensors is summarized, and the underlying sensing mechanism is explained.*

Keywords*: nuclear tracks, membranes, biosensors.*

Introduction

In the past two decades or so it turned out that nuclear tracks in thin polymer foils may be useful for constructing biosensors. More than half a dozen different strategies had been developed for that purpose [1]. Our group favored the "product enrichment" strategy [2-4]. Here, the strong confinement within narrow etched ion tracks hinders the products of the reaction of enzymes (the latter ones being bond to the etched track walls) with indiffusing biomolecules from easy escape through the track openings, so that they enrich within them. This product enrichment determines the minimum detectable biomolecule concentration and therefore has to be enhanced as much as possible to allow for the detection of even slightest traces of bio-matter.

On the one hand this is enabled by increasing the etched track length L, as the enrichment scales with L^2 [5] (i.e., by using thicker carrier foils for the tracks, or by tilted angle ion irradiation). On the other hand, as well decreasing track radii r decreasing ratios of product's to analyte's diffusion coefficients $D_{\text{prod}}/D_{\text{an}}$ are helpful [5]. However, these attempts encounter physical or technical limitations. The maximum possible track length L is determined by the energy of the used energetic ions, hence is limited by the capability of the available ion accelerator; the track radius r is limited by the space necessary to accommodate a minimum number of reaction products so that

accurate transmitted currents can be recorded, and usually the ratio D_{prod}/D_{an} cannot be influenced at all in a given system.

1. Strategies for improved biosensors

Another possibility is to retard the enzymatic product loss by inserting obstacles into the tracks. As impermeable (or at least semipermeable) membranes within the tracks are the most efficient obstacles, we put our attention onto them. Membranes can be accommodated, in principle, at two different places, either at their entrances or somewhere in the center of the tracks (Fig. 1). Both strategies are pursued by us.

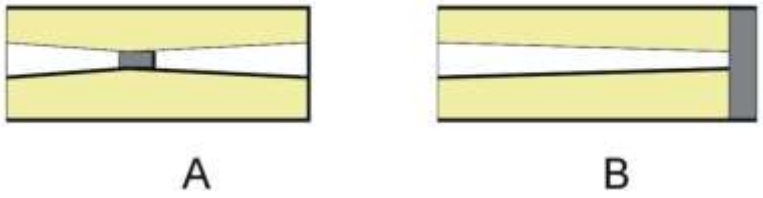

Fig. 1. The two possible configurations to accommodate membranes in etched tracks for biosensing; a) membrane in between two etched track halves, b) membrane on exit of one long track

1.1. Membranes on track surfaces

If etched tracks are open only on one side, diffusional loss of enzymatic reaction products occurs only on one side, hence the loss is reduced by a factor 2. Further, one has to take into account that the maximum track length L from which the products can escape is now twice as long as earlier, as in the previous case the maximum diffusion pathlength up to one of both surfaces was only $L/2$. This means – taking into account the square root relation of diffusive effects with the diffusion pathlength – the diffusional loss is now $(1/2)^2 = 1/4$ of the previous case. Taking both, the loss of one track exit and the enhanced track length into account, we arrive at a factor of $1/2 * 1/4 = 1/8$ by which the diffusional loss is reduced and hence the sensitivity enhanced.

Such membranes can best be produced by evaporating a thin film (that is resistant against etching) onto one side of the irradiated polymer foil before etching and etching the tracks from the opposite foil side only. Other possibilities are to stop the etching shortly before breakthrough occurs (this is difficult as during etching the accurate thickness of the remaining polymer is not known), or by depositing a film onto the nanoporous foil after etching (also very unsafe as the closure of all pore openings is not easily guaranteed).

1.2. Membranes in etched track centers

The second possibility led to the idea to couple two chemical-topological reactions with each other in dynamic nanometric confinement so they would be compelled to react in a peculiar way towards membrane formation. The first reaction is that one of an etchant (NaOH or KOH in our tests) with a swift heavy ion-irradiated polymer (here: polyethylene terephtahalte, PET). This reaction preferentially dissolves the radiation-damaged regions in the polymer so that nanopores emerge. If a track-containing foil is etched from both sides, the two emerging nanopores will meet each other in the center so that continuous pores emerge.

{NaOH – $AgNO_3$} reaction: If, however, this etching reaction is coupled with another one that produces impermeable precipitates, central membranes might be formed. One example is to use silver ions for that purpose. For that sake, the previous etchant has to be removed on one foil side and replaced by $AgNO_3$, whereas etching continues on the other foil side. Upon etched track breakthrough, the silver ions will react with the etchant according to:

$$AgNO_3 + NaOH \rightarrow AgOH\downarrow + NaNO_3$$
$$2AgOH \rightarrow Ag_2O\downarrow + H_2O$$

Under favourable circumstances, that silver oxide will act as a membrane, thus separating the etched tracks into two compartments [6].

Different reaction channels. Unfortunately, not every attempt to produce central membranes within etched ion tracks in this way is successful as the membrane formation processes strongly depend on the boundary conditions of the coupled reactions. More detailed studies [6] have shown that three basic parameters − the etchant concentration, the etching temperature and the applied voltage − determine the outcome of the confined reaction. Whereas both the etchant concentration C and temperature T determine the etching speed, the applied voltage U determines the electric field strength across the emerging Ag_2O membrane, and consequently the ionic migration mechanism through the latter. Slow etching at low C and T with not too small applied voltages U leads to a gradual build-up of separating membranes of good quality, whereas at lower U, the membrane quality is somewhat worse. It becomes even more worse − as seen from the decreasing membrane lifetimes − for low C but high T and/or U. High C at whatever T and U do not lead to any membranes but to complete track filling with the silver oxide.

If silver ions enter the reaction kinetics only after etchant breakthrough has already been achieved, membrane formation is no longer possible as the Ag_2O precipitates cannot anchor on the walls any longer. Instead, they are just swept out from the etched tracks by the usual nanofluidic mechanism. This limits the time up to which the etchant in one track side can still be replaced by the silver salt solution [7].

{LiCl – NaF} reaction: Another possibility is to use LiF membranes for that purpose, exploiting the reaction:

$$LiCl + NaF \rightarrow LiF\downarrow + NaCl$$

Again, under favourable circumstances that lithium fluoride will act as a membrane, thus separating the etched tracks into two compartments. In this case it is advantageous that the etching reaction needs not be interrupted as in the previous case, as both Li^+ and F^- ions can coexist with the NaOH etchant, without any premature precipitation occurring [8].

As compared with e.g., Ag_2O membranes, the advantage of LiF membranes in biosensor production would be that the latter ones can, in principle, be removed at any time later on by dissolution in much water, to connect both track compartments with each other again. This gentle strategy does not destroy any organic material deposited before in the etched tracks, such as enzymes. For contrast, dissolution of e.g., Ag_2O membranes in strong HNO_3 would also destroy previously deposited enzymes, thus rendering the production of coupled biosensors in this way impossible.

Also in this case the membrane formation is strongly limited to special sets of boundary conditions. In dependence of the mechanism which is just dominating in the special case, one also may find [8] –

* the formation of a protective LiF coating on the polymer surface that prevents its etching at all,
* LiF precipitation all along latent ion tracks, or
* long-lasting smooth ionic currents without any continuous track etching,

apart from the desired transient LiF membrane formation within the etched tracks.

2. Electronic properties of membranes

For Ag_2O membranes in etched tracks, detailed studies were performed to characterize them electronically, by applying low-frequency voltages $U(t)$ across the measuring chamber (including both the foil and the electrolytes) and determining the passing currents $I(t)$ [9]. Further, the corresponding $I(U)$ characteristics, the four pole parameters according to the electronic network theory, Bode plots and Fourier spectra were determined. This was achieved by means of a Velleman PCSGU250 pulse generator/oscilloscope combination. Fig. 2 shows an example for a) measured $U(t)$ and $I(t)$ curves, b) the $I(U)$ plots derived thereupon, c) the frequency dependence of the current amplitude (as given by the difference I_{pp} between maximum and minimum currents) and d) the frequency dependence of the corresponding phase shifts $\varphi(\upsilon)$.

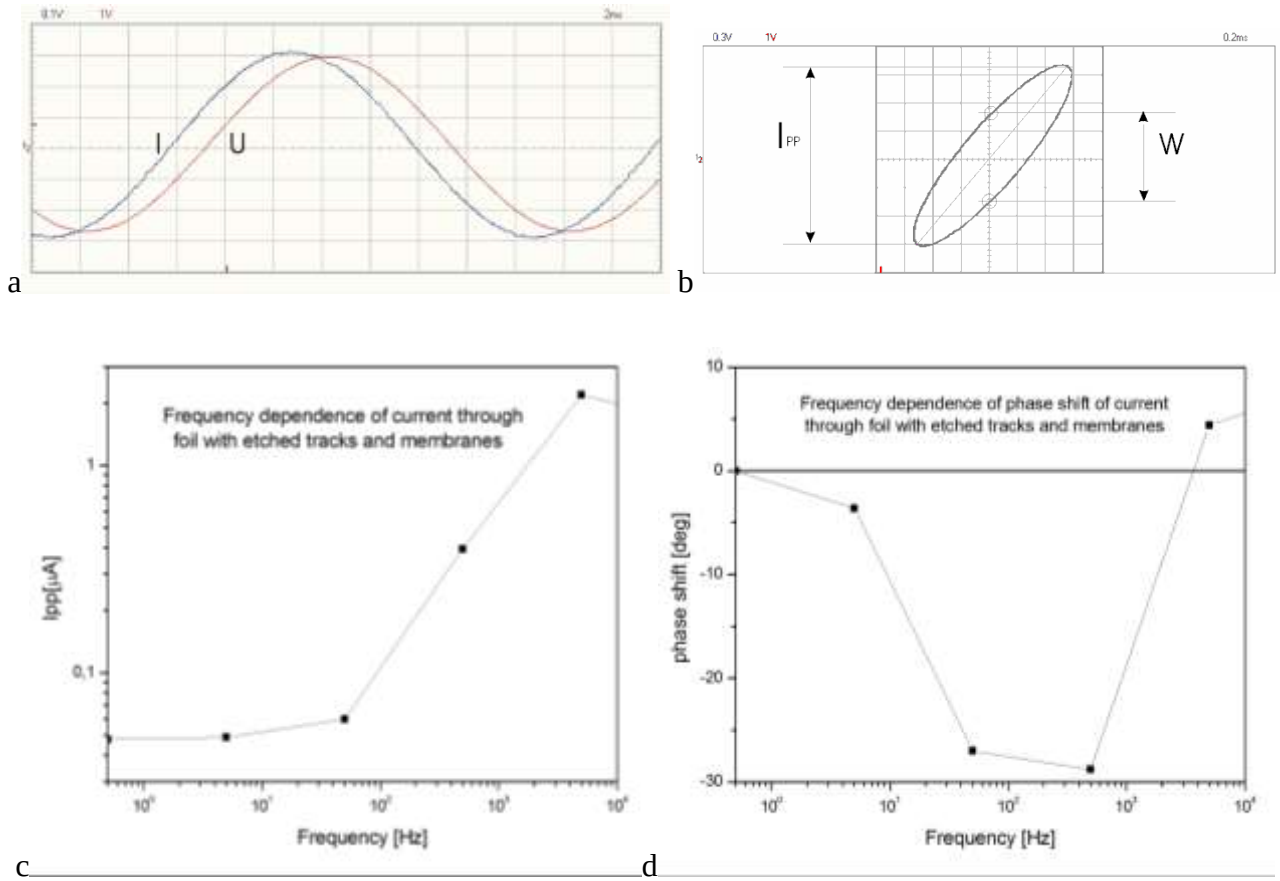

Fig. 2. PET with 5×10^7 etched ion tracks per cm^2 with central Ag_2O membrane in water; a) measured U(t) and I(t) curves and b) I(U) plots, both determined at 50 Hz. c, d) the frequency dependences of both c) the current difference ΔI(υ) (as defined in the text) and d) the phase shift φ(υ)

Whereas one can conclude from the comparison of the $U(t)$ and $I(t)$ curves Fig. 2a) directly as well the sign as the magnitude of the phase shift φ, this information is obscured in the $I(U)$ plots (Fig. 2b), insofar as the magnitude determines the width W of the emerging ellipses, whereas the sign is contained in the (usually invisible) rotation direction of the ellipses with time. Whereas the absence of any phase shift indicates purely Ohmic behaviour, negative φ indicate the presence of capacitive, and positive φ the presence of inductive effects. The latter ones can be excluded in the given case; occasional positive φ at higher frequencies could be attributed to instrumental artefacts and are therefore omitted here.

From Figs. 2c,d it is concluded that etched tracks with central membranes have a peculiar frequency dependence: whereas at 1Hz and below they behave like highly Ohmic resistances, some capacitive component emerges in an intermediate region of 100...1000 Hz range and vanishes again at higher frequencies. For contrast, ordinary etched tracks (i.e., without membranes - not shown here) show a dominant capacitive behaviour even at the lowest frequencies that gradually turns Ohmic with increasing frequencies. As expected, the presence of the membranes decreases the intensity of the transmitted currents through the etched tracks; only in the kHz range the intensities of tracks with membranes approach to those ones of tracks without membranes.

The fact that the currents transmitted at low frequencies (~Hz) amount to only about 1% of the higher frequency (~kHz) values (Fig. 2c), signifies that these structures act as low frequency filters. This is also reconfirmed by corresponding Fourier spectra of transmitted currents when using a multi-frequency alternating voltage as input signal (not shown here); low frequency current signals are largely suppressed. If the capacitive current response is exploited instead of the Ohmic signals (Fig. 2d), the membrane structures even act as band passes for the 50 to 500 Hz frequency range. In other words, the Ohmic response of sensors produced in this way is strongly tolerant

against hostile electrosmog stemming from low-frequency household, transport and industrial noise, and the capacitive reponse is additionally also deaf for high frequency electrosmog stemming from radio, TV, radar, microwaves and others.

To our knowledge, the complex four-pole parameters h_{ij} (i,j = 1,2) according to the electronic network theory have never been analyzed for ion track structures. This will, however, become important once such structures undergo industrial applications as e.g., biological sensors. The knowledge of h_{ij} will enable one to predict their behaviour in any electronic circuit. Here, h_{11} describes the short-circuit input impedance, h_{12} is the open-circuit reverse voltage transfer ratio, h_{21} is the short-circuit forward transfer ratio, and h_{22} the open-circuit output admittance. The results of the four-pole parameter measurements (a special measuring and evaluation procedure [9] allows for compensation of the above-mentioned instrumental artefacts so that these evaluations could be expanded up to 1 MHz) are depicted in Fig. 3. All parameters show a minute increase with frequency and some shallow curvature with a slight maximum at intermediate frequencies.

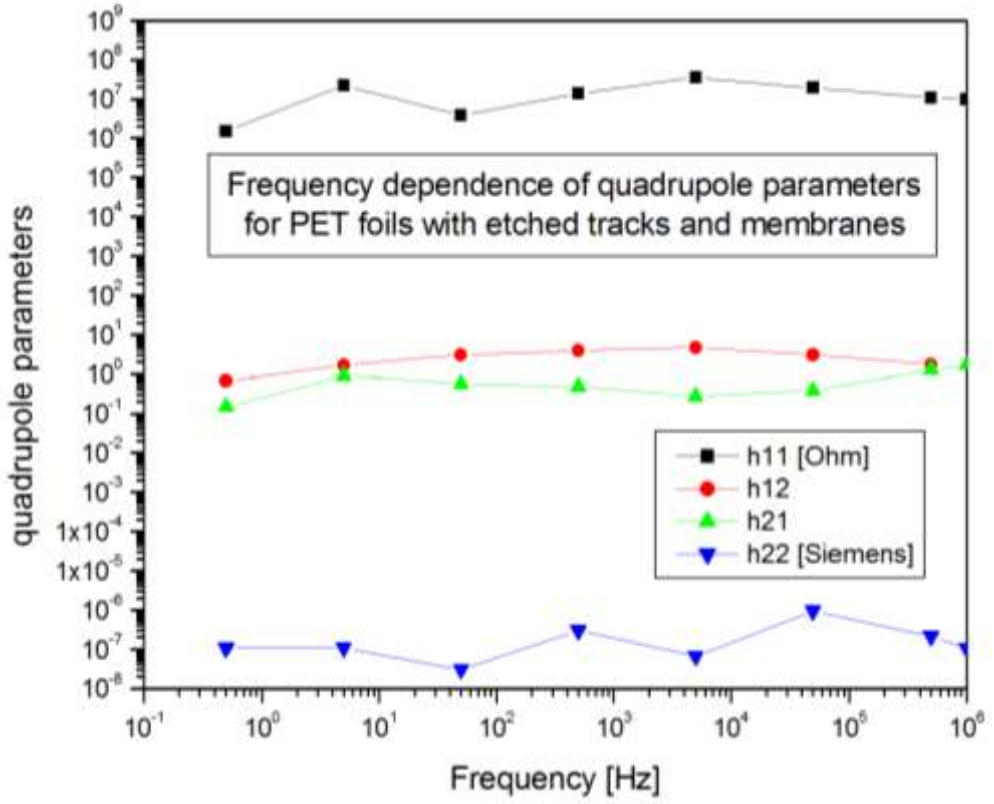

Fig. 3. Frequency dependence of the 4 four-pole parameters for PET with 5×10^7 etched ion tracks per cm^2 with central Ag_2O membrane in water

3. Nuclear track-based membrane biosensors with two functionalities

For practical applications, central membranes appear to be more interesting than surface membranes, as here one has two nanometric compartments on both membrane sides that can be filled with different materials, hence enabling one to detect simultaneously two different biomolecules. For the first tests we have filled, however, the same materials into both compartments [10]. The steeper the inclination of the current/concentration curves, the better the accuracy of the individually determined concentration values. This slope is steepest at the lowest frequencies in the ~Hz range and below, however, in this frequency range also the measured currents are smallest so that one soon arrives at the detection limit of the experimental equipment (in our case: ~1 pA). Therefore, as a compromise, we took ~10 Hz as measuring frequency in this work. We also preferred measuring the I_{pp} values as these signals are more pronounced than the W values according to Fig. 2b.

Fig. 4 shows the measuring result. The detectable concentration interval ranges from about 10 to 10^4 mM, hence is slightly less (due to detector saturation at higher concentrations) than that determined with previous simpler track-based sensor [2] without membranes (that ranged reliably up to 0.2 M). When comparing the sensor accuracies (as given by the curve's slopes) of the two concepts with each other, one recognizes that the membrane-containing sensors enable much more accurate sensing. Further, one notes that the concentration calibration curve decreases here with increasing glucose concentration, whereas the calibration curve increased in the previous cases.

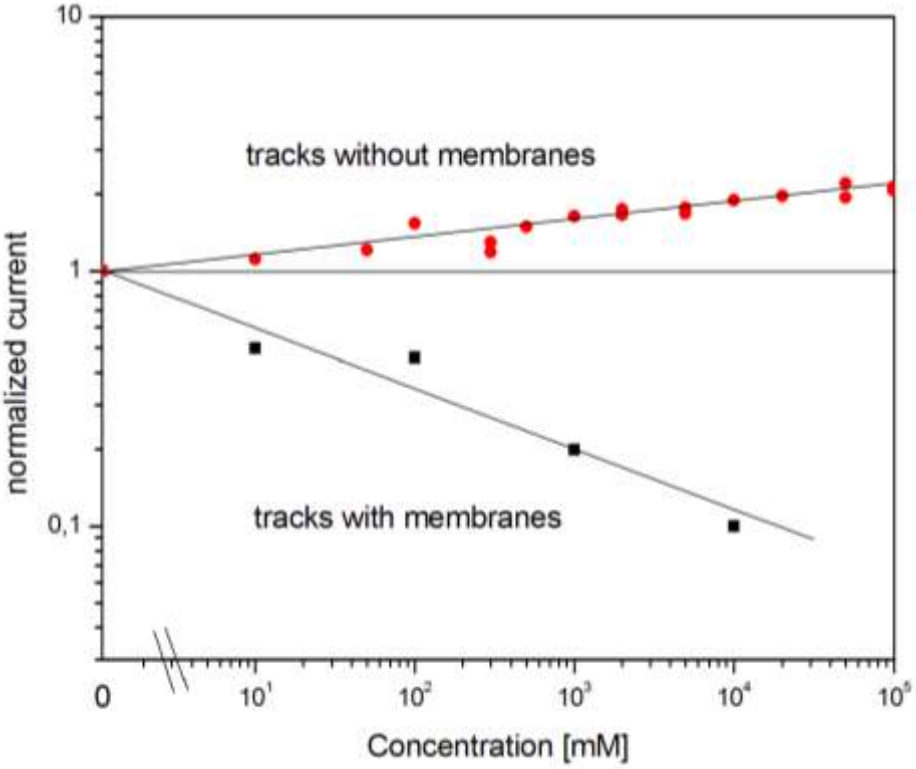

Fig. 4. Optimum calibration curves for glucose sensing, for two different sensor concepts – that one using etched tracks without (overtaken from [2]), and with membranes (this work). For the data evaluation of the latter, the differences between maximum and minimum current amplitudes at an applied frequency of 10 Hz were extracted from the measurement. For easier comparison, the total currents transmitted through the foils were normalized to unity for zero concentration. It is clearly seen that the membrane concept described here yields a calibration curve with much steeper inclination, hence with better measuring accuracy

These findings, and the fact that the tracks with membranes are much less transparent than tracks without membranes indicate that we deal here with a different sensing mechanism. A tentative explanation is illustrated in Fig. 5.

Step I (Fig.5, I-A and I-B): The indiffusion of the analyte into the tracks proceeds in both Cases A (without membranes) and B (with membranes) in the same way. There reactions with the attached enzymes (symbolized by thick red lines on the track walls) take place. If the formation rate of the reaction products (the small green circles in Fig. 5 stand for the negatively charged products - here: gluconic acid ions) is larger than their loss rate, they will accumulate and thus increase the track conductivity. (For the sake of simplicity, the counterions – here: protons – are not shown in the figures). This holds as well for Case A as for B (of course, in Case B one has to take into account the additional considerable membrane resistance).

Step II (II-A in Fig. 5): Application of an external test voltage leads to a shift of the product distribution within the tracks and to additional product loss in one direction. This loss is compensated on the opposite side by a reduced product loss.

II-B in Fig. 5: The presence of the membrane distorts that product distribution insofar as products on the left membrane side are prevented from migrating and thus accumulate in front of the membranes. Products on the right membrane sides are lost as ion Case II-A. Of course, the intrinsic electrical field being built up by the reaction products (here: GA^-) will attract some counterions (here: H^+) on the other membrane side, so that the region around the membrane gets polarized. However, the internal electrical field being built up by GA^- and H^+ is negligible as compared with the externally applied one.

Step III; III-A: If the externally applied voltage is inverted, the current intensity remains unchanged but flows into the opposite direction. III-B: Now, products on the right membrane side accumulate in front of the membranes, and those on the left side can escape.

Step IV: Now let us consider very high analyte concentrations, that led to high enzymatic product concentrations within the tracks. In the Case IV-A, this leads to higher recorded currents across the etched tracks. This means, the expected concentration calibration curve will have a positive slope. However, in Case IV-B the polarization stemming from the intrinsically built up electrical field will now no longer be negligible, but counteract strongly the external field, so that the overall electric field will decrease with increasing analyte concentration, and hence also the measured current. This is indeed observed.

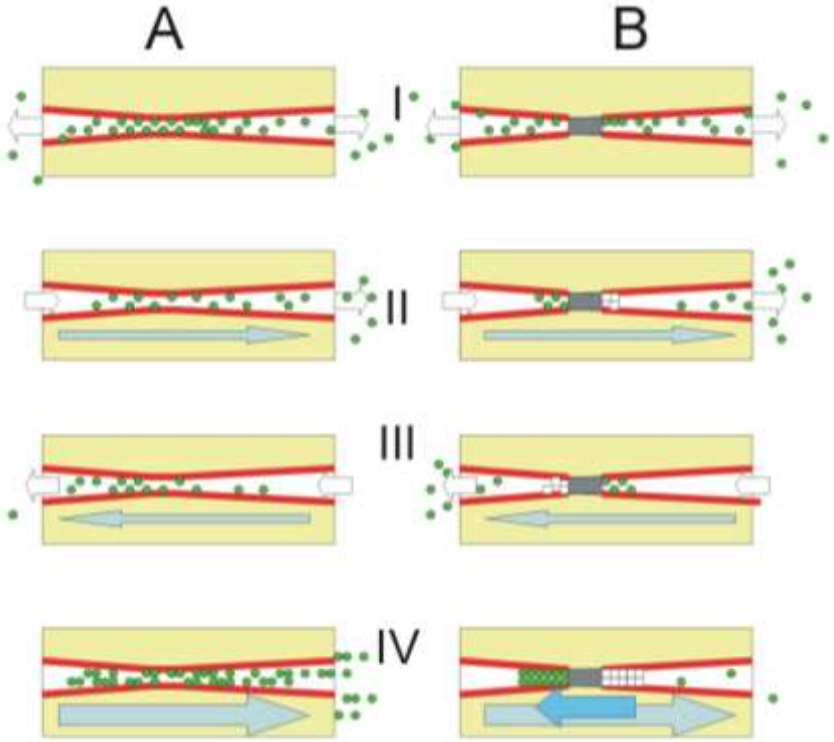

Fig. 5. Sketch of the tentative mechanisms for etched tracks a) without, b) with embedded membranes (grey sqares). I-A: analyte indiffusion promotes reaction with enzymes (symbolized by thick red lines on track walls) --> Product accumulation; charged products (green circles – in this example: negative gluconic acid ions) yield higher track conductivity. Part of the enzymatic reaction products lost by diffusion through the track openings. For the sake of simplicity, the counterions (here: protons) are not shown in A. II-A: Application of external test voltage (light blue arrow) leads to additional product loss. III-A: Inversion of external voltage leaves current height unchanged, but changes current direction. IV-A: Higher analyte concentration yields higher track conductivity. I-B: same behaviour as in I-A. II-B: Product accumulation on left side in front of membrane; product loss on right side, but counter-ions accumulate (white rectangles). III-B: same as II-B, but inverted. IV-B: High analyte concentration leads to higher accumulation of both products and counter-ions --> higher intrinsic electric field (dark blue arrow), counteracting the external field --> lower transmitted current

When evaluating the widths of the ellipses in the *I*(*U*) diagrams, hence when evaluating the capacitive effects of such sensors, one obtains quite similar calibration curves [10] which are therefore not shown here.

The electronic behaviour of foils with etched tracks which were closed on one side by a membrane is quite similar to that one of foils with tracks with central membranes. This is understandable, as primarily the electronic properties depend on the membrane thickness and material, but not on the membrane's position within the tracks. Also, the biosensing behaviour of foils with enzyme-clad tracks with surface membranes yields qualitatively no major difference to the results reported above.

Summary

It is shown that the insertion of membranes into etched ion tracks in thin polymer foils changes the sample's behaviour insofar as they obtain low frequency filtering properties. This is useful for suppressing low-frequency electronic noise which often deteriorates the quality of sensitive measurements. Best working conditions are obtained when using ~kHz AC voltages for measuring. Cladding the walls of membrane-containing etched tracks with enzymes transforms these samples to biosensors with better sensitivities than those ones without embedded membranes. The underlying reason is that the presence of the membrane enables a different sensing mechanism to act.

Acknowledgements

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Chapter 8. PERSPECTIVES OF MULTIFUNCTIONAL ION IRRADIATION-PRODUCED THREE-DIMENSIONAL STRUCTURES FOR BIOTECHNOLOGY

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Abstract. *New ion iradiation-based 3-dimensional structures are developed for biosensing and other biotechnological applications. On the one hand, this is accomplished by the further development of the swift heavy ion track technology and on the other hand by the combination of this technology with ion-beam based surface microstructuring techniques. Our goal is to produce a cheap universal generic micrometer-sized working platform with three-dimensional nanostructures and multifunctional properties for the analysis of biomaterials and cells, specifically mammalian cells and bacteria. This shall be done on one side by surface engineering of a thin polymer foil for the purpose of cell bonding, and by both bulk and surface engineering of the other foil side for the purpose of detection of small complex biomolecules.*

This platform should especially help obtaining more detailed information about cell intercommunication, by determining the spectra of secrets (specifically signalling molecules) of different types of cells. Vice versa, this platform could act as a sensor for different cell (specifically, as bacteria) types, by identifying them via their individual "biochemical languages". This knowledge shall help developing strategies for preventing quorum sensing of bacteria, with the aim to fight bacterial infections in an environmentally-friendly way.

Keywords*: ion tracks, microbeams, polymers, etching, cells, bacteria, biosensors, biomolecules, signalling molecules, quorum sensing.*

1. Ion irradiation of thin polymer foils

1.1. Ion tracks in thin polymer foils

Since the sixties of the last century it is known that energetic (*with at least ~ tens of MeV*) heavy (*with atomic masses being usually larger than that of Ar*) ion irradiation *("swift heavy ions", SHI*) of solids introduces very narrow *(~ some nm in diameter*) but long (*with ranges R of typically 10-100 μm*) parallel trails of damage in irradiated polymer foils, the so-called latent ion tracks. In this paper we restrict to thin foils (*with thickness d << R*) as targets so that the latent tracks always penetrate throughout all the foils.

The radiation damage along the tracks shows up primarily by the formation of radiochemical reaction products. Whereas the smaller ones readily escape from the irradiated zone thus leaving behind them nanoscopic voids, the larger ones tend to aggregate towards carbonaceous clusters [1]. The thus emerging structural disorder along the tracks modifies their electronic behavior. The newly created intrinsic free volume enables electrolytes to penetrate into the polymer along the latent tracks, thus forming parallel liquid conducting nanowires between the foil front and back sides. Upon proper design, the irradiated polymer foils may exhibit electronic properties that mimic bioelectronic functionalities, as they resemble somewhat biological membranes which also contain a number of parallel electrolyte-filled nanopores.

The carbonaceous clusters along the latent tracks might behave as obstacles for the smooth ionic current passage along them, upon application of a DC or low frequency AC voltage across the track-containing polymer foil. As a result, charges may pile up in front of them until the intrinsic electric field across them exceeds the breakthrough field strength. At that moment current spikes emerge [2] which eventually are associated with negative differential resistances [3]. As the spike height is decreased by eventually adsorbed surface layers, pulsating tracks can also be exploited for biosensing [4].

Foils with current spike emitting tracks are thought to mimic neurons. In a multitude of such tracks, the individual randomly emitted spikes synchronize themselves towards phase–locked oscillations within domains of typically 20-30 mm^2 size (*determined by the mean free pathlength of the charge carriers within one AC half period*) [5, 6] – similarly as they occur for neurons in the human brain, where their interaction results in the formation of brain waves. The frequency of these collective track pulsations is around 0.1-30 Hz [2], hence in an order of magnitude which is similar to brain waves. The presently available neural network theory describes the behavior of pulsating tracks at least qualitatively well.

The radiochemical changes along ion tracks, preferentially chain scissioning, make the ion track region vulnerable to being dissolved ("etched") by aggressive chemicals (*such as NaOH in the case of PET as polymeric substrate*) [7], thus transforming the original "latent tracks" into nanopores, the so-called "etched tracks".

Whereas etching of a track from both sides for a sufficient time leads to cylindrical tracks (Fig. 1c), etching from one side only for a sufficient time leads to conical tracks [8] (Fig. 1a). Compensation of this etching process on one foil side by in-diffusing neutralizer solution from the other side leads to much shorter etched tracks, the so-called "funnel-type" tracks (Fig. 1b) [9].

It is known since long that polymeric surfaces {such as polyethylene terephthalate (PTE, mylar) or polycarboante (PT, hostaphan, Makrofol) charge up negatively if in contact with electrolytes [10-13]. In case of cylindrically etched tracks, this will not have any influence on the movement of embedded ions as the electric field gradient within the tracks is zero, i.e. the tracks act as Faraday cups. However, in the case of conically etched tracks with non-zero electric field gradients, a unidirectional non-zero force acts on embedded ions so that they preferentially move into one direction. Macroscopically, such tracks, if embedded in electrolytes, exhibit current rectification [14] and consequently can be exploited to determine the sign of the charge of transmitted molecules.

"Funnel-type" tracks combine both the rectifying and spike-emitting properties [9]. Any material of interest for electronics (such as (semi)conductors or enzymes) deposited within the etched tracks or on their walls may transform the pore-containing polymer foils into either nanoelectronic devices or biosensors, respectively.

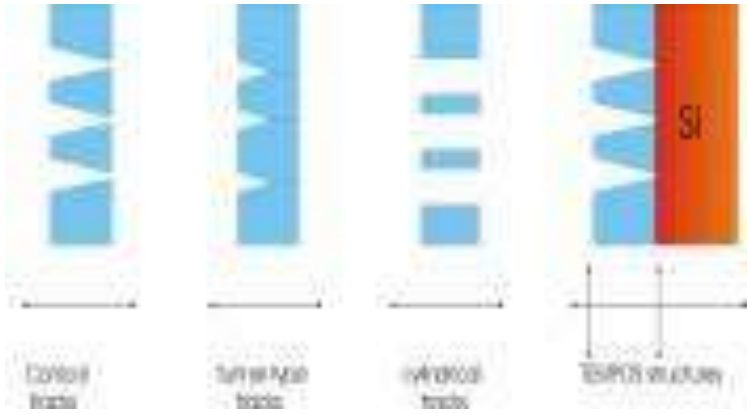


Fig. 1. Illustration of the variations of ion track etching. The tracks can be etched conically [7] (a), funnel-type-like [9] (b), or cylindrically (c), and they can be combined with a silicon substrate to form hybride structures with conventional Si electronics ("TEMPOS structures" [10-13]) (d). The principle directions of information exchange by ionic and/or electronic currents are indicated by the arrows

1.2. Microbeam-created surface structures in thin polymer foils

Ion microbeams of light low-energy ions (such as 3 MeV protons) allow one to deposit well-defined quantities of damage at precisely defined locations on, e.g. , polymer surfaces via coarse sample translation to the place of interest on the sample and subsequent beam scanning within a resolution of ~ 1 μm or so, according to the given irradiation requirements. The irradiation leads to some material´s removal by (physical and chemical) sputtering. Furthermore, the radiation damage created by this particle beam in the polymer´s bulk along the ion trajectories enables easy etchability. Thus, depending on the 2D-distribution of ion impact and on the duration of etching, three-dimensional topological surface structures of any desired shape (such as craters, holes, trenches, grids etc.) and depth can be created. Fig. 2 shows a first test example for the Porto Alegre microbeam.

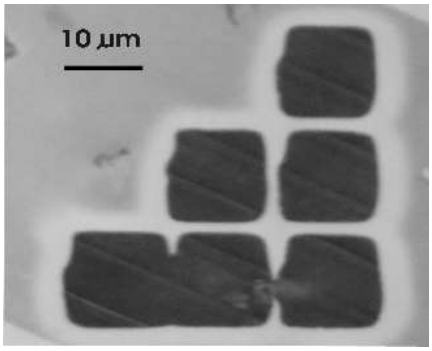
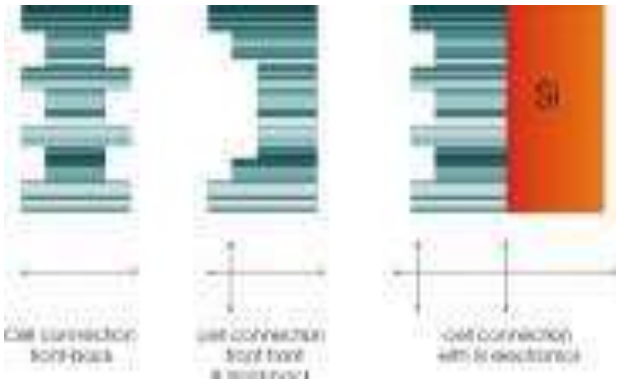


Fig. 2. First test experiment at the Porto Alegre microbeam facility. Image taken with an optical microscope under perpendicular illumination

These surface depressions are characterized by chemical material's modifications (due to the irradiation-induced enrichment of carbon and the formation of radicals, unsaturated and double bonds, etc. [1] and due to etching that removes surface contaminants and also exposes unsaturated bonds) that make them more biocompatible than unirradiated polymer surface regions. Furthermore, it is the altered surface topology that favors the bonding of living cells. For the purpose of this work – topologically selected cell bonding – surface structures with horizontal dimensions of typically ~10 μm and perpendicular dimensions of ~1 μm are required.

For $R >> d$ (as assumed for this work), the microbeam-created zone of radiation damage will extend throughout the whole foil. This means that, upon etching the irradiated foil from both sides, etched microstructures will emerge that are symmetric on both sides (as illustrated in Fig.3a). Assuming additionally that in our cases the beam scattering in the target is negligible (so that the irradiated structures do not broaden remarkably) and that the projectile´s stopping power does not change remarkably during its passage through the polymeric target foil (so that the etching rate of both foil sides is the same), then the microstructures on both sides will have identical shapes. This enables one to construct working platforms with a 1:1 relation of the microstructures on both sides. After connecting these identical microstructures by etched ion tracks with each other and after depositing different cells into the depressions on both sides, one has thus obtained an experimental platform for the direct and unambiguous comparison of these two cells with each other.



a) b) c)

Fig. 3. Illustration of the possibilities to combine etched ion tracks and microbeam-formed and etched surface patterns in polymer foils. The randomly distributed etched tracks in the foils are symbolized by the horizontal pattern. Etching of the microbeam-irradiated zones yields symmetric topological patterns on both sides (a), etching of one side only leaves the backside smooth (b). Last not least, combination of microbeam-patterend polymers with a Si substrate yields TEMPOS-like structures. Each microbeam-created pattern is thought to accommodate one cell or cell cluster. In a) two such cells on both foil sides are connected with each other by the etched tracks. Additionally cells in different front positions can be connected with each other by deep and narrow trenches (b), and cells can also be contacted with Si electronics via etched ion tracks (c). The principle directions of information exchange by ionic and electronic currents and/or biomolecules are indicated by the arrows

Focused ion beams might also fulfill the goals of this work, however in this case it has to be clarified in how far eventual gallium contaminations of the created structures might affect the behavior of the attached cells. In a later stage, when routine production of these platforms is required, it might turn out that sample irradiation with a wide ion beam through masks might be the better choice due to its greatest simplicity.

2. Present state-of-the-art of ion-irradiation-based devices

2.1. Present state-of-the-art of ion track technology

A large experience in swift heavy ion track technology has already been accumulated. On the one hand, this concerns especially the creation and investigation of ion-track / Si hybride nanostructures which form a principally new foundation for interfacing of conventional electronics and ion-track based electronic devices. Following this approach, novel multifunctional electronic devices with unique parameters (see below) have already been created that were hitherto unknown in electronics [10-13]. On the other hand, ion track technology has already been directed towards biosensing applications. Here a possible approach it to functionalize the ion tracks directly by

attaching organic or bioactive compounds (such as enzymes) to their walls. The authors have already obtained a number of results in this direction, specifically for glucose and urea sensing [15, 16]. The recent advances in this field allow monitoring and tracking of these biomolecules in areas such as environment, food quality and health. The presently developed ion track-based nanosensors provide high sensitivity, low power and low cost.

2.1.1. TEMPOS structures

In former experiments, SiO_2/Si and SiON/Si bilayer structures were ion-irradiated, etched and filled with (semi)conducting matter, to produce novel electronic devices with peculiar properties such as tunable rectification and tunable switching (flip-flops), negative differential resistances (NDR) accompanied by light emission, high resistivity against electromagnetic pulses (EMP), te capabilities for current amplification, self-oscillations if exposed to energetic irradiation, intrinsic capability for making logic AND and OR decisions, and sensing of physical (such as light, temperature, magnetic fields, etc.) and chemical (such as H_2O, NH_3, etc.) parameters (Fig. 1d) [10-13]. These structures were denoted by the acronym "TEMPOS" which stands for "tunable electronic material with pores in oxide on semiconductor". Later on this group was expanded towards bilayer structures made of irradiated polymers (such as Kapton) and Si. It is expected that, when the walls of the etched ion tracks of these devices are clad with first a conductive layer and then with, e.g. enzymes, one should be able to produce rapidly responding biosensors that are compatible with conventional Si electronics [17].

2.1.2. Track-based biosensors

Self-carrying ion track-containing thin polymer foils can be used as substrates for the construction of biosensors. The ion tracks can either be used as-prepared or after etching. Two approaches must be distinguished (see also Fig. 2 in [17]): Historically, the first strategy developed for track-based biosensing was the blocking of the transmission through a polymer foil containing a ***single etched*** track clad with an appropriate bio-agent, by a specific biomolecule, which increased the foil resistance [18, 19]. Whereas the advantage of this strategy is its extreme sensitivity by making single biomolecules detectable, its disadvantages are the need for highly precise pore preparation and the very low currents to be detected.

Therefore we developed a more robust approach which requires much less effort for sensor preparation and yields much larger currents for biosensing. The much worse detection sensitivity of that approach does not signify any drawback for practical applications as the minimum detectable concentrations C_{min} are still far below medical requirements C_{med} (for example, for our glucose sensors [15], $C_{min} \sim 10^{-5\ldots-6}$ M whereas $C_{med} \sim 5\times10^{-4\ldots-3}$ M). This could be accomplished by using a ***multitude*** (typically $10^{6\ldots9}$ cm^{-2}) ***of parallel etched*** tracks (each of them clad with an appropriate bio- agent, e.g. an enzyme) within a thin polymer foil, and by the simultaneous enrichment of ionic products of suitable (such as enzymatic) bio-reactions within these tracks due to their high confinement [15, 16, 20, 21].

A modification of the second technique is given by the adsorption of bio-reaction products onto the surface of foils containing ***many latent*** SHI tracks which leads to a decrease of the foil conductivity [4, 20, 22]. For more sophisticated systems, two or more such foils were placed in parallel. These systems were denoted as "Electronics with Electrolytes in Etched Tracks" (E^3T) as they form a new field of biomimicking electronics. Such systems have e.g., an inherent capability for being used for logic AND or OR decisions.

2.2. Present state-of-the-art of ion-beam based microstructuring

In the past decades, ion-beam based surface microstructuring techniques (by microbeams, focused ion beams and irradiation) through masks) gained an increasing importance. They allow one to introduce well-defined tiny areas of radiation damage into the surface of adequate substrates which upon etching can be transformed into 3D-surface microstructures. In numerous papers it was shown that such surface structures favor the bonding of biomaterials and living cells (including mammalian cells, bacteria and viruses) [23]. In this connection, even or evaporation of carbonaceous materials through masks [24-27] should be mentioned as a competitive technique.

3. A new platform for biotechnology

In this paper we present our new strategy how to apply the above-mentioned nuclear technologies – i.e., swift heavy ion track formation in polymers and their irradiation with ion microbeams – for the creation of advanced biotechnological devices. These new structures can be applied as well for sensing of biomolecules as of cells, including mammalian cells and bacteria. In this paragraph we summarize first the basic biological knowledge required for the proper outline of the new platform – i.e., the question how either biomolecules or cells bond to the polymeric base structures – and thereafter the platform itself is presented.

3.1. Adhesion of biomolecules and cells to polymers

In Ref. [15] a possibility is described how bimolecules such as enzymes can be bond successfully to polymers. The idea was to build a "molecular bridge" between the polymeric –COOH groups and the amino groups of, e.g.- enzymes or other peptides by using a combination of EDC and sulfo-NHS. Electronic examinations of the sensors created in this way indicated that enzymes could be attached to about 99% of all available –COOH groups. This recipe was found to work well for bonding of as well glucose oxydase [15] as urease [16], and it is expected to be applicable also to other amino- group containing peptides such as enzymes. Due to its efficiency, it was meanwhile also overtaken by other groups [28, 29], however without giving any adequate reference.

Concerning the adhesion of cells to polymeric surfaces, it is meanwhile known that usually there does not exist any universal straightforward recipe as all cells behave differently from each other. What is beneficial for one cell type might not have any influence on another. Especially, human and mammalian cells show behaviours different from those of bacteria, and there exist also differences in the behaviours of encapsulated and non-encapsulated bacterial strains. Nevertheless, some general rules-of-the-thumb have been established how cell adhesion and proliferation on polymeric substrates can be influenced positively. These can be by:

- chemical factors such as a) the choice of adequate biocompatible deposits, b) hydrophilicity, c) surface charges, d) chemical surface etching;
- energetic irradiations such as a) laser treatment, b) plasma treatment, c) low energy ion irradiation, d) high energy ion irradiation;
- topological 3D structuring of the substrate surface by a) mechanical techniques such as molding, casting, evaporation, electrospin-coating of nanofibers etc. and b) irradiation (e.g. by ion microbeams or laser irradiation) and eventual subsequent etching. In general, both nano-and microstructuring of the substrate influences the cell adhesitivity and other cell properties positively. The first possibility is most widespread in literature (~50%), followed by the second (~30%) and third one (~20%).

Another point concerns the cell arrangement on the substrate. If isolated cells are deposited on a planar surface of a synthetic polymer, they usually tend to connect with each other to form layered structures. For example, in the case of mouse NIH 3T3 fibroblasts, cell monolayers with moderate clustering and some empty space in between are the preferred cell arrangements for functional cells as they allow the cells some degree of cell mobility and proliferation. However, in the case of excessive clustering, they tend to die [30]. It is assumed that this tendency for clustering is supported by cell intercommunication via secreted signal molecules that allow the cells to know in which direction to move or to grow.

Therefore the optimum geometry for cell arrangement has to be found out for each special case. Whereas for bacteria, sites that can accommodate only one cell eventually might be adequate, for cells like fibroblasts or neurons they are not. In this case it is better to allow them for at least some limited degree of interconnection with each other (e.g. by arrangement in larger-spaced trenches or grids) so that they "feel comfortable" and yield regular signals instead of secreting stress hormones.

Anyhow, it is generally accepted that three dimensional multicellular organoids represent a more realistic tissue model as compared to isolated single cells lacking cell/cell contacts or conventional cell monolayers in 2D configurations. Therefore one should strive, as a rule-of-thumb, for cellular platforms that combine a spatially extended surface zone (as being produced

e.g., by a microbeam in the μm scale) with good surface functionalization (as being given by e.g., ion irradiation that yields structures in the nm scale, plus etching (thus creating etched ion tracks) and eventual additional attachment of cell-friendly materials, see the appendix).

3.2. The nature of the cell bonding

Usually, cell adhesion tests are performed in the simplest way, by making microscopic images of the deposited cells, after cautious cleaning the substrates with buffer solution. The classical mechanical adhesion tests cannot be performed here as in this case the cells would get excessively stressed and subsequently die.

Cells (i.e., both bacteria and mammalian cells) are highly dependent on proteins adsorbed onto materials surfaces to mediate their adhesion. These proteins are adsorbed spontaneously from biological fluids, e.g. cell culture media and body fluids, and include mainly the extracellular matrix (ECM) proteins fibronectin, vitronectin, collagen and laminin. These molecules can be also synthesized by mammalian cells, and deposited on the material surface. Both bacterial and mammalian cells have specific adhesion molecules on their surface, which recognize and bind the mentioned proteins. In case of bacteria, these molecules are represented by a group of microbial adhesins, known as Microbial Surface Components Recognizing Adhesive Matrix Molecules (MSCRAMMs), e.g. the proteins Fnbp and Clf recognizing fibronectin and fibrinogen, respectively [31], the collagen-binding protein CNE [32] or the elastin-binding protein EbpS [33].

In the case of mammalian cells, the cell-matrix binding is realized through integrin and non-integrin cell adhesion receptors. Integrins are transmembrane glycoproteins which bind specific amino acid sequences in the proteins adsorbed on the material surface, such as Arg-Gly-Asp (RGD), recognized by most cell types. Non-integrin receptors are proteoglycan-based and bind either oligopeptidic ligands, e.g. Lys-Arg-Ser-Arg (KRSR) recognized by osteoblasts, or they bind saccharide ligands, e.g. galactose (for a review, see Refs. [34, 35].

The process of modifying the surfaces of the polymer is essentially to change the nature of the extracellular matrix protein interaction with the surface to mediate cell adhesion. For example, on highly hydrophobic surfaces (with water drop contact angles $\geq 100°$), the cell adhesion-mediating ECM proteins are adsorbed in rigid and denatured form which limits the accessibility of oligopeptidic ligand in these proteins by cell adhesion receptors. On the contrary, moderately hydrophilic surfaces (e.g., contact angles from 50° to 80°) adsorb the ECM molecules in a more physiological geometrical conformation, which is advantageous for the receptor-ligand binding. However, highly hydrophilic surfaces (with water drop contact angle near 0°) do not allow stable adsorption of cell-adhesion mediating ECM molecules, particularly if the high hydrophilia is combined with motility of molecules (e.g., polyethylene oxide chains tethered on the material surface; [36], and thus these surfaces inhibit the cell adhesion galactose (for a review, see Refs. [34, 35]).

Different cell types may respond differently to the same surfaces which may be a consequence of cellular expression of adhesion molecules. For example, the amino acid sequences Tyr-Ile-Gly-Ser-Arg (YIGSR) and Ile-Lys-Val-Ala-Val (IKVAV) in laminin molecules are preferentially bound by integrins $\alpha_6\beta_1$ and $\alpha_7\beta_1$ on neurons. Similarly, the vascular endothelial cells bind preferentially the sequence Arg-Glu-Asp-Val (REDV), present in fibronectin, by their $\alpha_4\beta_1$ integrin receptors. Another example is that albumin, a blood serum protein which is preferentially adsorbed on hydrophobic surfaces, binds bacteria but not mammalian cells (for a review, see Refs. [34, 35]).

Restricted expression of integrins could possibly allow cell spreading but also cause progressive cell death due to apoptosis [37]. Some bacterial MSCRAMM adhesion molecules, e.g. CNE, can also interfere with the integrin-mediated adhesion of mammalian cells [32].

The cell survival times on a material surface depend on a wide range of factors, such as physical and chemical properties of the cell adhesion substrate, species, age and state of health of the cell donor, cell type and subsequent cell culture conditions.

As for the physico-chemical properties of the material surface, a good example is the surface wettability. With increasing the material surface wettability, the cell adhesion-mediating ECM

proteins are adsorbed in more physiological conformations, but more weakly and less stably. Thus, at later culture intervals, when the adsorbed proteins "hold" a relatively high number of cells, these cells can spontaneously detach from the material surface, and if they are anchorage-dependent (mammalian cells), they undergo apoptosis due to the deprivation of their adhesion. For example, human osteoblast-like MG 63 cells in cultures on relatively highly hydrophilic nanocrystalline diamond films terminated with oxygen (contact angle about 20-30°) detached on day 5 after seeding, while the cells on more hydrophobic hydrogen-terminated NCD films (contact angle 85-90°) remained stably adhered, although they formed a confluent cell layer or even multilayer [38, 39]. Similarly, rat vascular smooth muscle cells on more hydrophilic polystyrene samples irradiated with 5×10^{12} F^+ ions/cm^2 detached spontaneously in 1-2 weeks of cultivation, while on less hydrophilic samples irradiated with a higher dose of 5×10^{14} F^+ ions/cm^2, a confluent cell layer was stable for at least six months. In addition, polymers irradiated with the higher dose contained more conjugated double bonds between carbon atoms and showed a higher electrical conductivity [40], which might be another factor supporting the long-term survival of cells on the material.

As for the cell origin, the cells derived from phylogenetically more advanced donors (e.g., humans) are usually less viable than the cells from animal donors, i.e. they have a shorter life span, are more prone to senescence and have a limited number of cell divisions and population doublings. Similarly, the cells from younger donors are more viable than from the older donors of the same species. Also sex- and health-related differences are important. For example, vascular smooth muscle cells form male rats showed a higher turnover and spontaneous cell detachment and loss than the female cells. Similarly, a higher cell turnover and higher tendency to apoptosis was observed in cultured VSMC derived from spontaneously hypertensive rats (for a review, see [41].

Last but not least, the cell culture conditions are of a great importance for the cell survival. These conditions include the cell seeding density, time of cultivation, amount and composition of cell culture media, intervals between the medium changes, and the use of conventional static or dynamic cell culture system. The latter system (with circulation of cell culture media) improves the cell metabolism, nutrient & waste exchange and provides the cells with mechanical stimulation, which improves the cell functioning (for a review, see Ref. [35]).

3.3. Conversation between cells; Quorum sensing

The release of various compounds from bacteria to their environment has often an essential functional role as a means for overcoming host-defense mechanisms, allowing colony proliferation and facilitating bacterial communication. This means, the study of these secretion products enables one to understand the bacterial behavior and as a consequence, enables one to develop new strategies against their proliferation and growth. As each bacterium emits a unique spectrum of secretion products, their analysis enables one to detect individual bacteria species unambiguously. Many bacteria regulate their diverse physiological processes in dependence of their population size. For this sake, their intercommunication is an important strategy for them to survive in a host. Bacterial cell-to-cell communication utilizes small diffusible signalling molecules, which the bacterial both produce and perceive. There is a direct relationship between the concentration of chemical signaling molecules and that of the bacteria present in a given environment. As the bacteria couple gene expression to cell density, the signalling molecule concentration is a measure for the bacterial abundance and hence determines their behavior. Isolated bacteria try to hide themselves by avoiding excessive emission of products that would trigger the host´s immunization reactions, and rather protect themselves from e.g., antibiotics by slime production. However, when the signalling molecules reach a critical threshold, the "quorum" [42], the bacterial population as a whole modifies its behavior by synchronization to become able to act as a single unit and to yield a response that, in the case of malign bacteria, might induce diseases. "Quorum Sensing", QS, is hence the bacterial strategy for optimum surviving in a host.

The quorum sensing (QS) signals of Gram-negative bacteria are usually N-alcylhomoserine lactones (AHL) and in Gram-positive bacteria they use to be oligopeptides such as cytolysin. In addition, both Gram-positive and Gram-negative bacteria produce a family of signaling molecules known as autoinducer-2 that they employ for their communications [43]. Several bacterial species

produce the same AHL though in each it may be used to regulate different biological processes. The role of AHL in many important bacteria has still to be clarified. The communication within and between mixed bacterial communities is often yet unknown [44]. Though the levels of (meanwhile well-examined) AHLs in biological matrixes usually seem to correlate with the health status of the subjects, it appears that also other (much less examined) chemical signaling molecules such as the oligopeptides and AI-2 contribute to the role of quorum sensing in bacterial disease [43]. However, in addition to the important role of QS for causing bacterial infections, also QS-deficient bacterial strains are often still capable of causing infections [45]. As QS regulates several processes including bacterial pathogenicity, the monitoring of the QS molecules present in biological fluids may be a useful analytical tool as biomarker in monitoring of bacteria-related disease activity and managing the patient's health. [46]. Bacterial pathogenic attacks can well be prevented by interfering with the bacteria's AHL signal system. This is used e.g., by plants that possess strategies to produce their own metabolites such as signal mimicks, signal blockers, signal-degrading enzymes, and/or blockers of AHL-producing enzymes that interfere with the bacterial signaling.

It is this strategy which belongs to the presently still hot topics of medical research, in the hope to suppress (or falsify) bacterial communication by so-called inhibitors and thus to block bacterial transition towards their aggressive conformation also in humans. Higher levels of these biomolecules are nowadays often produced with transgenic plants [44]. In general, detection of QS is much easier than its prevention. QS detection is possible either directly, e.g. by liquid chromatographic (HPLC) or indirectly, e.g. by voltammetry. Real-time and precise indirect QS detection usually requires an engineered bacterium which responds to QS with some kind of marker, usually luminescence, and is useful for the evaluation of synthetic QS inhibitors. Indirect QS measurement can be performed on wild type (natural) bacteria, given that one of the molecules biosynthesized upon QS activation is redox active. This is detected through voltammetric scans as a change in current as a function of applied potential.

Karatuna et al. [45] mention that the standard disk diffusion method in biology does ***not*** reflect the susceptibilities of bacteria within a biofilm. This may be due to the embedding of AHLs in the bacterial slime which strongly retards their diffusion coefficient. Therefore, bacterial whole-cell- based biosensing systems (i.e. the analysis of whole bacterial cells secreted from the body) were developed for the detection of AHLs (the signal molecules of gram-negative bacteria). They have, however, some drawbacks insofar as the procedures necessary for sample preparation are lengthy and tedious and the overall duration of the analytical process is long. Also, multiple samples cannot be analyzed in one analytical run [47]. Here, our ion beam-based bacterial platform (described below) may show up as an attractive alternative concept. Sample preparation is straightforward as the cells need only to be attached to the polymeric working platform, and multiple samples can be analyzed in one analytical run, by attaching them, e.g.to different parallel platforms as illustrated in Figs. 4 and 5. An open question remains, however, still at present, how the retarding action of slime onto the mobility of Quorum sensing molecules can be overcome in the experiments.

3.4. Description of the platform for biotechnology

Based on the above summarized knowledge on optimum cell adhesion to polymeric surfaces and molecule secretion from cells, we designed structures with polymeric foils wherein micrometer-scaled microbeam-created large surface depressions exist on one foil side, and where nanometer-scaled etched ion tracks connect ***exclusively*** these depressions with the foil backside, Fig. 4. In accordance with e.g., [24-27], the depressions are optimized for preferential bonding of selected cells to them. Whereas for depressions of typically ~10 μm size and > 0.5 μm depth, individual cells can be accommodated comfortably, larger sizes enable the accumulation of cell clusters of predetermined size. The fixation of cells and/or cell clusters at well-defined sites on a biocompatible substrate enables one to have these cells easily accessible for more detailed examinations and to perform experiments of greater accuracy and unambiguity than usually obtained by conventional large-size biological experiments in petri shell cultures. In this way, it is also possible to connect each individual cell by contact stripes for electrophysiological experiments.

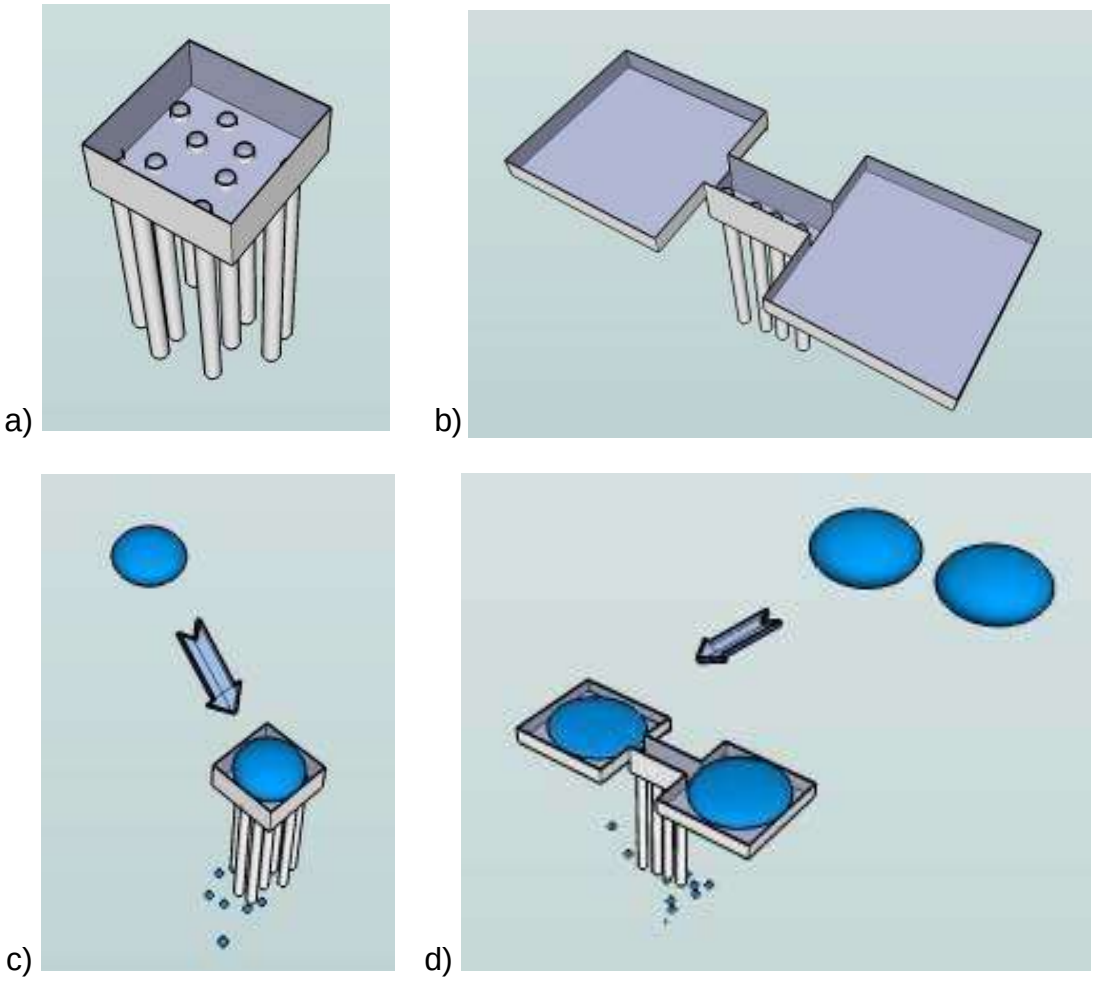


Fig. 4. Sketch of the structures used a) to extract secrets from cells, b) to intercept quorum sensing. In both cases, microbeam-created surfaces depressions exist that are connected by etched ion tracks with the foil backside. In b), an additional narrow and deep trench connects the surface depressions, and only the trench itself is connected with the foil backside via etched ion tracks. c), d) Cells arriving at the platform are bond inside the depressions at the foil surface. Their secrets are extracted via some (chemical or physical) potential gradient applied through the etched ion tracks towards the foil backside c) either directly or d) during the cell's (biochemical) information exchange

Due to their large (>> 1 μm) size, non-helical cells (including mammalian cells and bacteria) do not have any chance to penetrate through the tracks, however their secretion products do. As the cells tend to fill out all the space within the depressions (especially if these depressions have circular shapes), they thus shield the underlying ion tracks largely from the solution within which they are embedded, so that there is little chance for this medium to leak towards the other foil side. In order to concentrate the cell population to the depressions only, cell bonding is avoided outside them by deposition of adequate cell-rejecting materials there (for a summary of such materials, see the appendix).

The distribution of the cells on transparent materials such as our polymeric platform is visible in their native state, i.e. without staining. In the case of reduced or no transparency (such as given in liquids such as blood), the cells are visualized from the foil backside by fluorescence staining, e.g. with a combination of Texas Red C_2-Maleimide, which stains the proteins of the cell membrane and the cytoplasm, and Hoechst #22242, which stains the cell nuclei. Another combination is Alexa Fluor 488 for cell membrane and cytoplasmic proteins, and propidium iodide for the nuclei. Specific molecules in cells (e.g., integrins and integrin-associated focal adhesion proteins,

cytoskeletal proteins, secreted proteins) are visualized by immunofluorescence staining using specific monoclonal or polyclonal primary antibodies and secondary antibodies conjugated with a fluorescence marker. Fluorescently stained cells on the materials are observed in a fluorescence microscope (e.g., Olympus) equipped with a digital camera and connected with a computer with an image analysis system. Cells on irregular surfaces (i.e. rough, patterned, pseudo-3D surfaces) or cells on three- dimensional porous or fibrous scaffolds will be observed in the confocal microscope, which is able to perform series of parallel Z-sections and to summarize these sections into a 3D reconstruction of the cell-material complex focused at all levels. Very important in the confocal microscopy is the possibility to observe selectively the cell-material interface, which is not possible in a conventional microscope. Confocal microscopes will be also used for special imaging techniques, particularly the technique of Second Harmonic Generation (SHG), used for imaging of native collagen secreted and deposited by cells on the material surface [48].

The cells on the material are also viewed by scanning and transmission electron microscopy in order to observe more deeply the details of cellular morphology (cell extensions, their relation to the material surface morphology, cellular organelles, cytoskeleton etc.). In case of need, also HPLC and mass spectrometry are applied. In addition, cell-secreted biomolecules can be also visualized by immunofluorescence staining and quantified by an enzyme-linked immunosorbent assay (ELISA), protein electrophoresis and immunobloting and flow cytometry. The gene expression, i.e. the presence of mRNA encoding specific protein molecules secreted by the cells, can be determined by a real-time polymerase chain reaction (real-time PCR).

In principle, the basic concept to separate two compartments of a vessel from each other by a porous membrane from each other is not new; it has been realized, e.g. by Berg and Turner already in 1990 [49]. However, their aims and objecvtives were quite different from ours. They used microchannel plates of 1/2 mm thickness and about 1 cm^2 area with pores of diameters as large as 10 or 50 μm to separate the two compartments from each other, for studying cell diffusion effects.

Also the work of Giselbrecht et al. a few years ago [50], who applied microthermoforming of ion-irradiated and etched polymer films, is not directly comparable with our approach. The disadvantage of this approach is that the thus created porous compartments are usually in the order of a few 100 μm size, hence more than an order of magnitude larger than what we use to create by microbeams. In this way, bonding of single cells or of assemblies of only a few cells (specifically bacteria) at well-defined places is impossible. Further, this approach leaves the foils transparent everywhere, whereas in our concept the foils are transparent only at the place of the microbeam-created surface microstructures and hence are selective to the cell-emitted secrets only. This means, our approach is more advanced insofar as it allows a much better geometrical definition of the cell positions and of the origin of biomaterials migrating towards the cell-free foil side.

The etched tracks are expected to serve as channels for extracting the cell secrets to be analyzed towards the foil back side. Their accumulation there in a clean solution (buffer or water) enables their background-free examination. The efficiency of the connection between the cells and the other foil side is determined by the overall track cross section, i.e. by the number of etched tracks and their diameter. For a track density of ~ 4×10^6 tracks cm^{-2} and for cell-containing microstructures of ~10 μm diameter, in average 4±2 tracks are expected to end up in each microstructure. For etched track diameters of ~100 nm, the overall cross-sectional area of all these etched tracks is in the order of 3×10^{-10} cm^2, hence amounts to 0.03% of the whole microstructure area. For higher track densities in the order of ~1×10^9 tracks cm^{-2}, each microstructure will be hit by about 1000±30 tracks which occupy 0.75% of the whole microstructure area.

The concept of extracting secrets from cells within microstructures on polymeric surfaces through a number of etched ion tracks should yield some enrichment of these secrets within the tracks. First, secrets emitted from the cell towards the foil direction cannot escape into the opposite direction (and be diluted there in the ambient solution) as they are blocked by the cell itself. This can be further supported by putting a suitable chemical or physical potential gradient across the membrane towards the foil backside. Also, the cross sectional area of all etched tracks being in contact with the microstructure is only a tenth or so of the microstructure area itself so that all

secreted molecules will be enriched within the tracks by this order of magnitude. On the other hand, the diffusion coefficient of the secreted molecules within the confined etched tracks will be lower than their bulk diffusivity, hence their concentration will be further enhanced due to their smaller migration speed. Last not least, as the potential energy of the secreted molecules within the tracks is lower than that of the molecules within the microstructure (due to the smaller track dimensions), there exists a potential gradient from the cell within the microstructure towards the etched tracks. This also favors the molecule´s enrichment within the tracks. Last not least, deionized water or a buffer solution on the opposite foil side will reduce the background in detection of the extracted signal molecules. According to Figs. 4 and 5, the above-described platform can be modified towards various possible configurations, specifically for cell conversation studies.

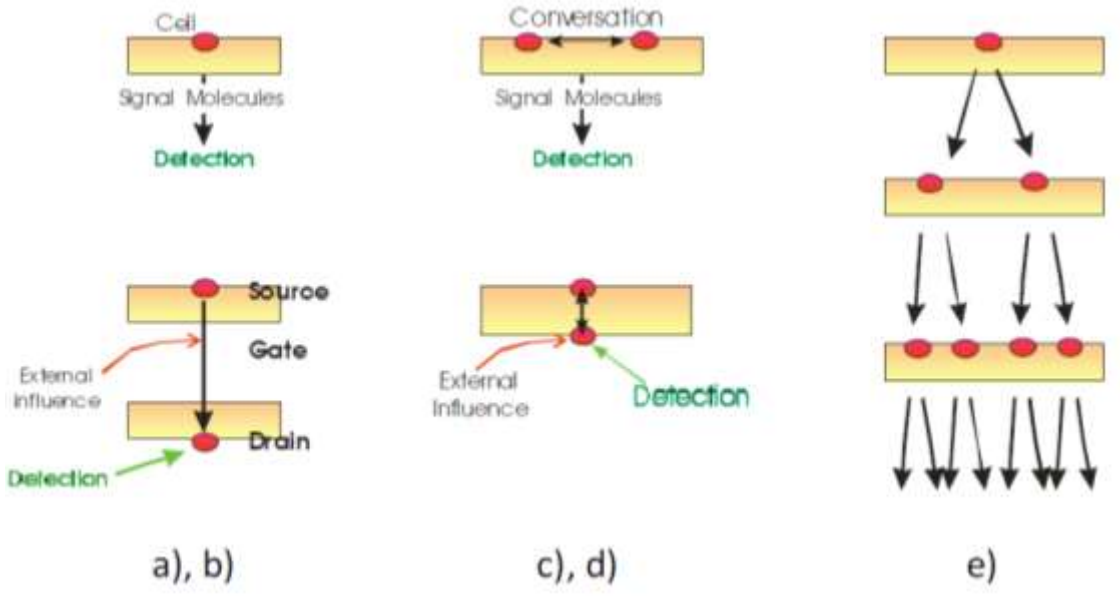


Fig. 5. Possible arrangements of cells on microbeam- and etched track-containing structures with one (a, c, d) or more (b, e) foils. Cells can communicate with each other either on the same foil side (c, e), or with cells on the opposite foil side (d). It is even possible to combine two or more parallel platforms with each other, to obtain transistor-like (b) or multiplier-like (e) biomimetic arrangements

In the first of the above-mentioned cases, cells bond to the surface depressions at some distance to each other (to prevent direct cell-to-cell contact) may be connected with each other by narrow microbeam-produced trenches (so narrow that blocking by another bacterium settling there is prevented) on the same foil side (Figs. 3b and 4). Due to three surfaces in close vicinity, these trenches are regions of strongly lowered surface potential energy which hence will attract many (neutral or charged) of the molecules secreted from the cells. The migration of these secrets, specifically of QS signaling molecules, within trenches with parallel walls will always be direction-independent (thus allowing "normal" bi-directional conversation A↔B between two cells A and B, even with charged signal molecules). However, specifically in the case of charged signaling molecules [51] and conically shaped trenches (i.e. with large openings at one cell side and small openings at the other cell), a specific direction of migration will be favored as such trenches act as rectifying devices, similarly as in the case of cylindrical and conical tracks. Hence in this case the conversation of two cells via charged signal molecules will be only unidirectional: A→B, so that the usual decision-making of cells will be blocked or at least hindered. Application of appropriate potential differences between the two cells A and B (e.g., by inserting electrodes in their vicinities will then compensate this blocking: A↔B or even invert it: A←B.

Etched ion tracks connecting (only!) these trenches with the opposite foil side can then serve

as channels to either intercept the cell-borne signalling molecules of the cell conversation by pumping them to the opposite foil side or to introduce other molecules such as inhibitors. This can be enabled by applying a small pressure gradient across the substrate foil. There they can bedetected by luminescence or by attaching (cyclic- or differential pulse-) voltammetry or even a chromatographic system directly behind the foil. To allow for precise cell communication tests, this cell conversation can be switched on or off by depositing thermo-or pH-responsive gels onto the ion track walls that can either open or close the track in dependence of the foil temperature or pH value [52, 53], respectively. The enhancement of both the signal and the signal/noise ratio are favorite conditions for performing the routinely applied biochemical techniques (such as the high-performance liquid chromatography (HPLC), mass spectrometry, voltammetry, luminescence, Fourier transform infrared spectroscopy and combinations of the above-mentioned techniques such as the liquid chromatography-tandem mass spectrometry (LC-MSMS) [47] right behind the tracks. In the case of voltammetry, this can be done by depositing a gold electrode directly on the backside of the irradiated membrane prior to its etching.

The combination of our platforms (i.e. of foils containing both ion tracks and microbeam-created surface patterns) with Si substrates towards TEMPOS-like or transistor-like structures should enable, in principle, cell conversation with conventional Si electronics (Fig. 3c). Cell-Si hybrid systems have previously often been studied, however the toxicity of Si prevented rapid rapid progress in this field. Therefore it is suggested to hermetically seal in this case the biological part from the electronic one. This can be accomplished by accommodating the cells as described above in a bio-friendly polymeric / carbonaceous ambient, and connecting the cells with the Si via the etched tracks, the latter ones being clad by a thin layer of conducting polymers at the Si interface, to prevent any direct cell-Si contact. In this way, also Si surface corrosion and hence deterioration of its electronic properties can be prevented.

Though the results and suggestions described here essentially refer to the commercial non-degradable polymers such as polyethylene terephthalate (PET), polycarbonate (PC), polyimide (PI) (and eventually also to polysilanes and polysiloxanes) only, it should be kept in mind that there exist other polymers (such as chitosan, polylactite, polystarch etc.) that gained an increasing importance in the past years due to their biofriendliness and biodegradability. As it is not yet clear at present in how far the above-mentioned biosensing concepts could be applied to these polymers as well, such an examination should be performed urgently.

Further, it will have to be found out in how far etched ion track-containing foils could eventually be replaced by thin layers (either self-carrying or as layers on Si supports) consisting of nanoporous Al_2O_3 or TiO_2 [54]. Their much higher pore density (~10^9 to 10^{12} cm^{-2}) appears advantageous as compared with etched tracks in polymers with typically ~10^6 to 10^9 cm^{-2} track density, but the easy solubility of the amphoteric Al_2O_3 in both acidic and alkaline media and the worse biocompatibility as compared with track-containing polymer foils might outweight these advantages.

4. Perspectives of future ion-irradiation-based devices

In the following we shall list a number of fields where, to our understanding, microbeam-modified and/or ion track-containing structures (including the platform described above) can be usefully applied for future advances tasks in biotechnology. These are studies on a) mobility of biomolecules, b) advanced ion track-based biosensors, c) real and artificial neural networks, and d) Quorum sensing (QS).

4.1. Studies on the mobility of biomolecules

4.1.1. Determination of diffusion coefficients of biomolecules in confined environment

Recently, a simulation of the enrichment of enzymatic reaction products in long (length l = ~10 μm) and narrow (diameter $d \geq 10$ μm) etched ion tracks was made. The simulation yielded a bunch of curves for the product's depth distribution, each curve being described by a certain diffusion coefficient ratio $D_{product}/D_{anaylte}$ [55]. However, the true value of the reaction product enrichment in confinement remains still unknown, due to the lack of knowledge of both the diffusion coefficients of the analyte diffusion $D_{anaylte}$ into the enzyme-clad tracks and the

outdiffusing enzymatic reaction products, $D_{product}$. Therefore these values have to be determined.

For this sake, an etched track-containing polymer foil shall divide a vessel into two compartments A and B. At zero time, compartment A is filled with an analyte solution (e.g. glucose) whereas compartment B contains a corresponding enzyme solution (e.g. glucose oxidase, GOx). After the time $t_{analyte}$, first analyte traces will arrive in compartment B where they react with the enzyme, so that the conductivity of *B* is increased. From both $t_{analyte}$ and *l*, $D_{anaylte}$ can be determined.

At the time 0, compartment A is filled with both analyte and enzyme solution, which start immediately reacting with each other. The enzymatic reaction products being conducting, the conductivity of compartment A will start increasing. After a time delay t_{prod}, also the conductivity of compartment B will increase. From both t_{prod} and *l*, $D_{product}$ can be determined.

In a similar way, also the enzyme´s diffusion coefficient D_{enzyme} in the etched tracks can be determined: At the time 0, compartment A is filled with the enzyme solution, which diffuses through the tracks towards compartment B which is filled with the analyte. Once the enzyme solution arrives there after a time delay t_{enz}, it triggers the enzymatic reaction that yields ionized reaction products so that the conductivity of compartment A starts increasing. From both t_{enz} and *l*, D_{enzyme} can be determined.

4.1.2. Track-based sensors for helical bacteria

There exist some classes of parasitic bacteria with distinctive helical morphology (of body thicknesses around ~0.15…0.5 μm and spiral lengths of ~5…10 μm), such as spiroplasma (e.g. E.coli) and spirochetes (such as Borelia and Treponema) that eventually initiate severe diseases such as meningitis and syphilis. All of them can move with considerable speed (typically ~3-10 μm/s), however with different migration mechamisms. Whereas spiroplasma bacteria move via kinks in their helical structures that migrate through the whole bacterial body (resulting in a laterally swaying movement and thus requiring more lateral space), spirochetes exhibit a purely longitudinal movement (due to flagellates incorporated in the space in between their double membranes, resulting in zero additional lateral space required) [56].

The peculiar elongated structures of these bacteria allow them to pass along nanopores such as etched tracks. This is known since long and it is used routinely for their isolation by filtration, usually applying Millipore foils of 0.3 to 0.45 μm diameter for that purpose [57, 58]. Combination of filtering with agar medium containing rifampin facilitates the direct isolation of spirochetes, due to the inhibitory effect of rifampin for bacteria other than spirochetes [59]. Further addition of adequate nutrient solutions [57] on the nanopore exit side enables rapid multiplication of the transmitted spirochetes. Thus, the combination of ion track filters (clad on one side with rifampin and on the other side with nutrient) with any unspecific bacteria sensor (such as those described in Ref. [22]) should yield efficient spirochete sensors.

In spite of the widespread use of ion track filtering for helical bacteria and of extensive studies on their propagation mechanism [56], to our knowledge their mobility in very close confinement (i.e. << 0.5 μm) was never determined in detail (there exist, however, bacterial diffusion examinations within much larger capillaries of 10 and 50 μm size [49]). This shall be done by measuring their mobility through tracks etched up to different diameters in the range of the helix diameter. Such measurements will give interesting insights into the limitations of the movement of nanometric helical structures under confinement. It will further help distinguishing spirochetes from spiroplasmas in the above-described sensor concepts.

4.1.3. Biosensors that exploit synchronized pore blocking

As was mentioned above in chapter 2.1.2, a disadvantage of the pore blocking strategy for biosensing [18, 19] is that hitherto, only one single pore can be used for that purpose. This is because in the case of multiple etched tracks, all signals that emerge upon passage of biomolecules through the tracks would overlap in an undefined way due to their uncorrelated statistical arrival in time. If, however, these signals could be synchronized, one could expand the pore blocking strategy to foils with a multitude (e.g., 10^6 to 10^9 cm^{-2}) of parallel etched tracks, which would improve the applicability of this strategy greatly, as the transmitted currents would now increase from the pA

range (for one single pore only) towards the range of μA to mA.

At least for charged biomolecules, this should be indeed possible. According to Fig.6a, the preparation of the corresponding polymeric substrate requires in this case (apart from the usual irradiation with swift heavy ions, etching from one side only (to obtain conical tracks) and depositing an adequate biomaterial (e.g., an enzyme) onto the pore walls), just one more step: the subsequent evaporation of two thin layers, a metallic one (preferentially with a metal of little toxidicy) and an insulating one. The evaporation should take place under tilted angle to the polymer foil surface, to avoid excessive materials deposition into the etched track cone. Then the evaporated metal layer has to be connected with an electrical contact EB. The whole polymer foil is inserted into a measuring chamber according to work [8], thus separating two electrolytes in two compartments A and C from each other which are connected by two contacts EA and EC. The compartment A contains the analyte biomolecules to be measured.

As long as no voltage is applied, nothing will happen, except for slight thermal diffusional losses of the biomolecules through the tracks (step A in Fig. 6b). When only the potential of EB is lowered below EA and EC, the positive biomolecules start accumulating at the open electrode metal at the track entrances which are thus slightly blocked (step B). When in the next step C, the voltage at electrode EC is lowered below EB, the aligned particles start migrating synchronously through the tracks, thus leading to collective total pore blocking if they are bond to corresponding biomolecules deposited at the pore wall tips. Blocking will continue forever, until the particles are released by a high voltage pulse applied between EA and EC. In the case that the particles are not bond to the cone tips, they will leave the tracks immediately after their synchronous passage (as shown in the graph). When the positively charged biomolecules start migrating from EB to EC, the potential at EA is equalized to that one of EB, so that the negative biomolecules are no longer attracted by the electrode EA. If in the subsequent step D, the potential at EA is lowered beyond that of EB, all negatively charged biomolecules will align at EB, so that in the next step E, synchronized emission of all negative biomolecules will take place.

If required to obtain higher accuracy, this sequential synchronization of the pore blocking of positive and negative biomolecules can be repeated many times. In the case that the sizes of both the positive and negative molecules differ greatly from each other (e.g., in the case of an acid where one has a big negative molecule and a tiny proton), the first step of the sequential measurement (passage of the proton) will not yield pore blocking at all, whereas the second one (pasaage of the big acidic molecule) will do. This means, one has an additional tool at hand to control the feasibility of this approach, by determining the size ratio of both differently charged biomolecule partners.

Apart from the availability of charged biomolecules and the synchrony in pore blocking, another basic precondition for the feasibility of this method is that all ion tracks must be etched under ***identical*** conditions, in order to arrive at identical pore openings. As was shown recently [60], etching of a multitude of tracks is ***no*** self-synchronizing process and thus leads to some scattering of track diameters. Therefore, close attention has to be drawn to efficient stirring during ion track etching, to enforce synchrony in track etching as only this asures equal nanopore sizes.

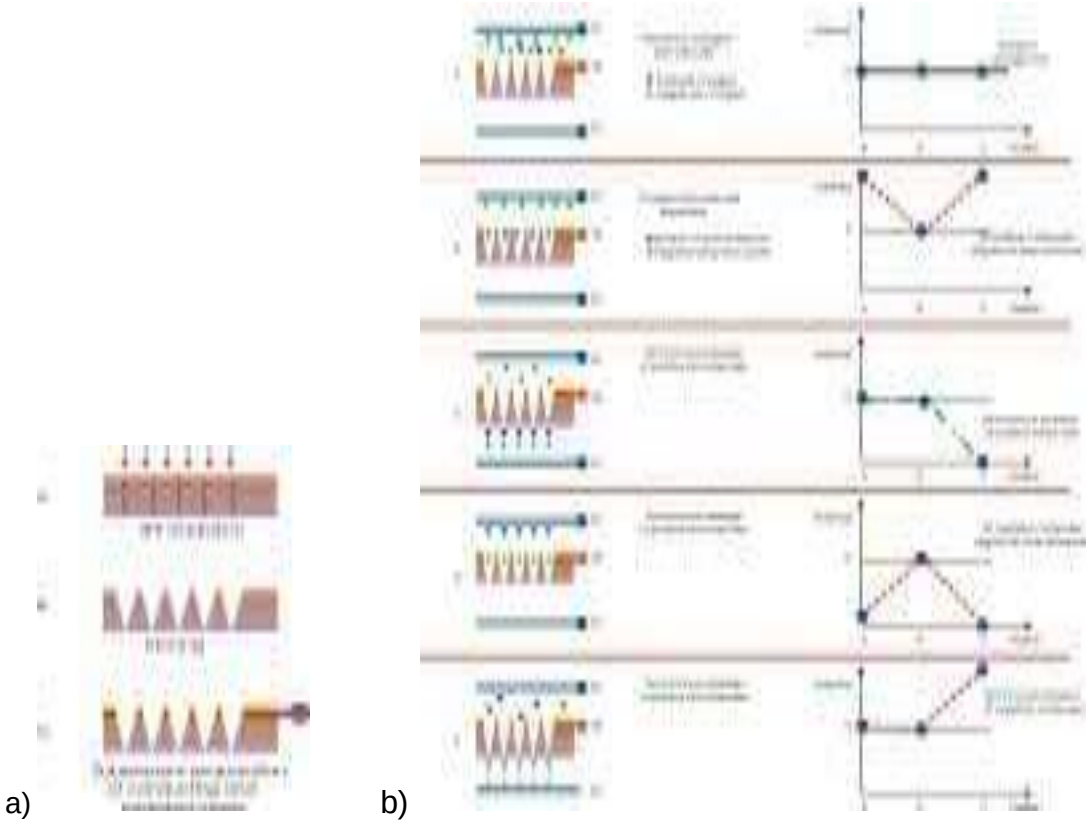
In the case that the biomolecules to be examined are bacterial secrets, an additional layer of Polydiacetylene (PDA) may be deposited optionally onto the foil backside, which will undergo as well a conductivity change as an optical change upon the arrival of the secreted molecules. PDA is a linear conjugated polymer with alternating double and triple bonds that exhibits a color change from blue to red upon its change in the structure from diacetylene to butatriene. This change can be initiated by interaction of PDA with, e.g. bacterial secrets (Gram positive bacteria: membrane peptides and toxins, Gram negative bacteria: endotoxins) [61]. Due to its conductivity, any changes in PDA configuration also lead to conductivity changes.

4.2. Progress in ion track-based biosensing

4.2.1. Ion track-based biosensors

The range of biomolecules which can be detected by our already existing ion track-based nanosensor platform shall be expanded to more biomolecules of interest such as ethanol, peroxides, dinitrophenol and cholesterol. As this sensor type is expected to be not only suitable for detection

and analysis of biomolecules in liquids but also of volatile organic compounds in gases, correponding tests shall be performed. The biosensors shall be modified towards portable tools for in-situ chemical analysis in terrestrial and space applications.



a) b)

Fig. 6. The synchronized pore blocking concept. a) overview of the production steps of the corresponding sensor substrate (irradiation, etching and deposition of contact layer and insulation, b) the cycle of applied voltages at the three electrodes EA, EB and EC, and the corresponding positions of both positive and negative biomolecules within the track sensor

Furthermore, there exists the possibility of self-enhancing the biosensor's response, when combining track-based biosensors with pH-responsive gels [52, 53]. Some of these 'intelligent' materials exhibit swelling which is highest for alkaline, and lowest for slightly acidic media. If in such a sensor a suitable analyte (e.g., glucose or urea) is detected, its acidic enzymatic reaction product will initiate the gel's condensation within the etched track, whereby the now larger available etched track volume will enable an enhanced reaction product accumulation, which in turn will promote the further gel's volume reduction.

The efficiency of simple ion-track-based enzymatic sensors can be improved by combining several sensor steps S_1, S_2, S_3, … S_n either sequentially or in parallel. The sequential steps can either contain sensors with identical enzymes: $S_1 = S_2 = S_3 = \ldots = S_n$ or different ones. A recent test of the first example with identical sequential urea sensors indicated indeed that the overall sensing sensitivity is roughly proportional to the number of foils [62].

The other case, sequences of non-identical sensors, applies to enzymatic reaction chains. If all intermediate products are detected properly, the overall measuring accuracy (being the product of the accuracy of all the intermediate steps) increases greatly. Such a system can be realized e.g., by placing several nanoporous foils sequentially behind each other (Fig. 7), the track walls of each foil being clad by the enzyme that is responsible for the corresponding step. A simple example is enzymatic glucose sensing which is a two-step process: D-glucose + O_2 + GOx D-glucono-lactone; D-glucono-lactone + $H_2O_2 \leftrightarrow$ gluconate$^-$ + H^+. There exist still other cases of enzymatic reaction chains where sequential steps are absolutely required to obtain the necessary sensitivity required to

detect usually highly diluted trace molecules such as pesticides or toxic gases. In this case, possibly all the steps can be united within single ion tracks each. The assembly of several sensors to one complex unit enables one to perform simple logic AND / OR tasks. Suppose that several different biomaterials M_1, M_2, M_3, … M_n in one analyte solution shall be sensed simultaneously, then one can bond the corresponding different enzymes to the walls of different ion track within one foil. If it is only of interest whether any one of these biomaterials exists in the analyte solution (i.e., the logic OR: M_1 v M_2 v M_3 v… v M_n), a spatial separation of the regions with individual enzymes is not necessary. If, however, the exact knowledge is required which one of the biomaterials is available (i.e., the logic AND: $M_1 \Lambda M_2 \Lambda M_3 \Lambda \ldots \Lambda M_n$), a spatial separation of the regions with different enzymes towards individual parallel compartments is required.

a) b) c)

Fig. 7. Illustration of the possibilities to vary and combine latent track-based structures (a) for biosensing. They can be combined sequentially to form sensor cascades (b), and different foil areas can be connected with each other to enable contact of different neural network domains with each other (c). The horizontal pattern symbolizes the parallel latent tracks in the foils. The principle directions of information exchange by ionic currents are indicated by the arrows

4.2.2. Use of living cells for biosensing

Many less common enzymes are very difficult to produce and consequently very expensive, so that their application in future biosensor mass production is prohibitive. In this case, a simple but elegant alternative would be, a) to grow the corresponding enzyme-producing cells within the microbeam-produced groves of our universal platform, b) to extract their special enzymes through the etched ion tracks below these cells, and c) to subsequently fix these enzymes as usual onto the walls of etched tracks in a neighboring polymeric foil which d) thereafter acts as biosensor for the desired analyte.

4.2.3. Time-integrated biosensors

In some cases, it is of interest not only to measure the concentration of a biomolecule in a sample at a given time, but also the biomolecule's integral concentration during a prolonged time interval. In a recent feasibility study it was shown that this can be accomplished by, e.g. the irreversible accumulation of materials (e.g., Ag that acts as marker for the given biomolecule) resulting from a suitable enzymatic reaction [20]. This strategy shall be further expanded towards the production of first demonstration sensors.

4.2.4. TEMPOS-type biosensors

In a former work, the possibilities of expanding TEMPOS structures for biosensing were already evaluated in detail [17]. Since it has become known that also carbon nanotubules and metal oxide nanowires can be grown within etched ion tracks [63], the possibility arose to functionalize them by attachment of biosensing materials. This is a hitherto yet untouched task that appears to be a promising alternative for biosensing. It combines somewhat the presently separate fields of E^3T (hitherto preferentially used for biosensing) and TEMPOS structures (hitherto preferentially used as ion track electronic devices) to a universal one, thus “marrying” biosensing and electronics with each other.

4.3. Real and artificial neural networks

4.3.1. Neural networks

Neurons tend to form pronounced long membrane-clad channels, the so-called dendrites and axons, for well-directed and secure (i.e. interception-free) information exchange among each other. It should be examined in how far such dendrites and axons might also be able to grow through etched tracks. If that is the case, one could force neurons (accommodated in microbeam-formed surface groves on thin polymer foils) to align their dendrite and axon network along pre-determined ion track patterns, for controlled study of their interactions. Eventually, the dendrite and axon networks could be designed so that their synapses are all exposed on the foil backsides, to make them easily accessible for external manipulations.

In this connection, also fibre-growing cells might be of interest; one could try here to accommodate the growing fibres within etched tracks, to obtain information about the degree of maximum spatial confinement that growing fibres still can tolerate.

4.3.2. Artificial neural networks

Spikes emitted from different latent tracks upon application of an external AC voltage with frequency ν can synchronize only if the charge clouds emitted from these tracks meet each other within a certain frequency-dependent time interval [2]. Due to the limited pathlength that ions can travel during that time, the domain size within which synchronization can take place is also limited. In previous experiments [2] these domain sizes were found to be in the order of typically ~ 10 mm^2. In larger samples, uncorrelated overlapping of different domains leads to more random spike emission. Distant domains in large sample can, however, be forcefully synchronized when connecting these domains by additional wires (Fig. 7c). This synchronization process could be manipulated by replacing the wires by electronically active elements; as a result different oscillation patterns could be tailored. In the case of different local biomolecule concentration in front of the polymer foil, different neural network domains will emit different synchronization patterns; the study of their differences will enable one to draw conclusions about the variations in biomolecule concentration.

4.4. Quorum sensing studies

In the simplest case, the existence of bacterial secrets can be detected by a non-specific bacterium detector such as described in [64]. Here, the bacterially-secreted compounds interact with agar-embedded {phospholipid / chromatic polydiacetylene (PDA)} nanoparticles which initiate both blue-to-red colorimetric changes and fluorescence transformations.

4.4.1. Bacteria sensor

If the surface patterns are created by sufficiently energetic microbeams in thin foils, etching the foils from both sides will create two exactly opposing surface depressions, linked with each other via etched ion tracks (Fig. 3a). Such configurations are of interest as they allow for studies of the details of conversation between two cells in different environments via the diffusing signal molecules. As every bacteria species possesses its own signal molecules spectrum, the detection of spectra of unknown bacteria should allow one to determine unambiguously that bacterium species. In other words, such experimental arrangement serves as a bacterium sensor.

4.4.2. Applications of cell conversation studies

It may be difficult to develop an easy way to directly detect AI molecules other than by HLPC. Perhaps an enzyme of antibiotic context would work, which degrades a pathogenic product produced as a direct consequence of QS activation [15, 16]. This strategy would somehow resemble our previous approach to construct glucose and urea biosensors.

Another option would be working the other way around: Pathogens secrete enzymes such as proteases, which break down proteins. The detection of such protein destruction products would be specific for such enzyme and hence for the pathogenic bacterium to be detected. The necessary suitable proteins (eventually fixed to a substrate) could be added to the buffer at the foil's detection side. Prevention of QS appears to be much more complicated than QS detection. If by applying a potential one might see a direct effect on QS, this could eventually be a way for QS control. This possibility should be checked by a test experiment.

The information exchange between two cells can take place via the liquid or the gaseous phase, depending on whether the cell-containing foils are embedded in liquids or whether they are dry. In both cases, the configurations suggested by us should enable the unambiguous proof whether some chemical or physical information exchange takes place between two cells or not, as the cell-containing foils can easily be arranged in a way to hermetically seal the two sides of the foil from each other. (In former experiments with petri dishes, there remained always some ultimate doubt whether not accidential contaminations of the cell culture under observation might have led to the corresponding observations.) Hence, if one cell shows new reactions (e.g. sudden antibiotic resistance of cells that were not resistant before) upon the presence of another opposing cell with adequate properties (e.g. of a cell with is already antibiotically resistant), it is clear that the new cell property could be acquired only via biomolecules migrating through the ion tracks as these are the only available connections between both foil sides.

There exists a long-lasting discussion with many contradicting results about the question in how far alternating electric fields emitted from microwave ovens, cellular phones, television sets, radar stations etc. might affect human health. Some time ago, also theoretical studies [65] were performed on that question, by calculating the force exerted on biologically important ions (such a K^+ or Ca^{2+}) that might allow them to migrate through cellular membranes into a direction different from that required for well-functioning cell metabolism. If such external electric fields would indeed create cell malfunction, this should also reflect in the spectrum of biomolecules released from such cells, which could be measured by the proposed setup.

In a similar way, also the influence of weak radioactivity, toxic materials, antibiotics, etc. on the spectrum of emitted signalling biomolecules can be examined, and thus give clues for cell resistivity against these adverse factors and for the thresholds when cell malfunction sets in.

Electrolytes in ***latent*** tracks in polymers serve for the transport of electric currents by the help of very small ions only (such as H^+, Li^+, OH^-, …). Upon etching these tracks, also larger molecules can participate in the cell´s information exchange, with the etched track diameter determining the size of the transmitted signalling molecules and thus stimulating them towards certain behavioral patterns. Fig. 8 gives a principle sketch of the production steps of such structures.

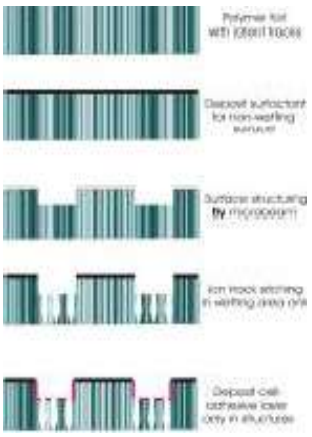


Fig. 8. Principle sketch for the production strategy of the structures recommended here. From top to bottom: a) SHI- irradiated polymer foil (i.e. foil containing latent tracks), b) same polymer foil with surfactant deposited on one side to obtain a non-wetting surface (this can be accomplished by, e.g. evaporation of a thin Teflon layer), c) structuring of the surfactant-deposited surface by microbeams. The irradiation destroys locally the surface´s non-wetting properties. d) Etchant attack of the as-prepared sample from the top side. Etching will occur only in wetting regions, i.e. at places where the previous microbeam attack had removed the non-wetting protective surface coating. Prolonged etching will transform the latent ion tracks into conically-shaped etched tracks which represent transparent channels towards the opposite foil side. e) Eventually, for enhancing the adhesion of specific cells in the microbeam-created groves, a corresponding layer can be attached to these groves

To find out whether the signal molecules are neutral or charged [66], it is sufficient to produce conically etched tracks between both sides of the polymer foil. Whereas the current/voltage characteristics are of Ohmic-type in the first case, it will be of diode-type in the second case. The direction of the current rectification measured in this case indicates the sign of the signal molecule´s charge. If, for some reason, this information is undesired when using samples with conically etched tracks, it can be readily “switched off” by bonding any positively charged molecules to the negatively charged polymer surfaces, thus neutralizing them.

To our knowledge, it is not yet known whether, below the QS threshold, the emission yield $Y_{QS,out}$ of QS molecules from a certain bacterium is virtually independent on the yield $Y_{QS,in}$ of arriving QS molecules from other bacteria, or whether there exists some correlation $Y_{QS,out} = f(Y_{QS,in})$ between both of them. This question can be easily tackled by using the biotec platform suggested here.

Finally, the study on the metabolism of specific anaerob metal-digesting bacteria by means of our proposed biotec platforms might be of interest, as these bacteria reveal unique surviving strategies in extreme environments as concerns lack of oxygen, extreme temperatures and the presence of toxins and heavy metals. Specifically, their ability to use electron receptors such as S, N, CO_2 and metal oxides allows them to process a large variety of different metal-bearing minerals. Their properties have already been applied for clinical diagnosis, wastewater treatment and decontamination of heavy metal exposure. One also can see good perspectives for their application in mining problems. The signalling molecules emitted by these bacteria might indicate the presence and eventually also the concentration of metal in minerals. This might open a way for producing ecological sensors.

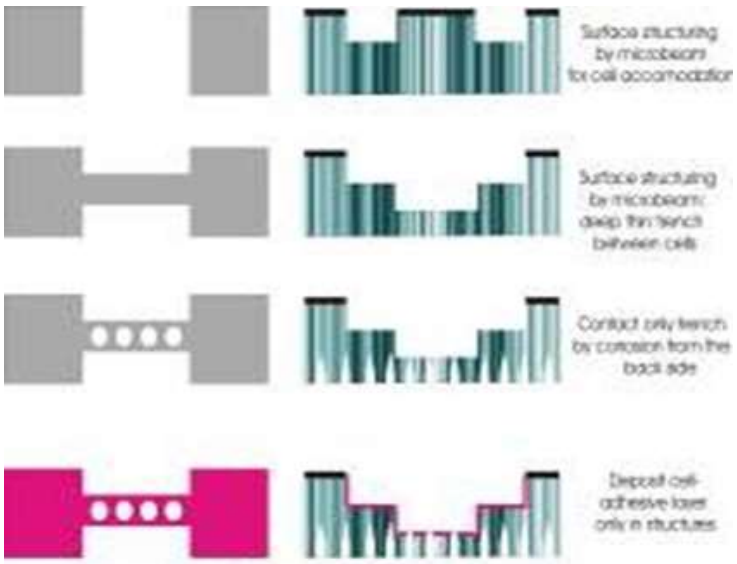


Fig. 9. The strategy to create structures for research on cell intercommunication. From top to bottom: a) creation of extended surface depressions by impact of microbeams onto surfactant-covered SHI-irradiated polymer foil. These surface depressions of whatever shape are expected to host the cells. b) formation of narrow trenches between these extended surface depressions (eventually with focused ion beams, FIB). The narrow width is expected to prevent cell deposition there; the greater depth of the trenches as compared with the neighboring depressions is expected to accommodate most of the messenger molecules emitted from the cells, to minimize their loss to the ambient. c) Etching from the foil backside will create a multitude of tracks with same lengths. The etching is stopped when the first tracks enable a connection to the trench, and before the cells in the depressions are also connected to the backside. d) Eventually, for enhancing the adhesion of specific cells in the microbeam-created extended surface depressions, a corresponding layer can be attached to these regions

4.4.3. Interception of quorum sensing molecules

QS molecules intercepted from the conversation channel between two bacteria (or bacteria clusters) can be used for their analysis or for studying the inter-cell chemical communication patterns, or by insert electric pulses or new signal molecules for disturbing the cell communication (Figs. 3b and 9). For such a case ion track etching from the back side might be preferential. Such experimental platforms might be useful for studying as well the communication between cells of the same species as between different species, to see how far the behaviour pattern of one cell type could eventually be transferred to another one. These platforms should also enable one to facilitate the development of strategies for preventing bacterial infections.

Summary

Both swift heavy ions and microbeams are valuable tools for nano- and micrometric 3D structuring of thin polymer foils. These possibilities are applied here for biosensing and other biotechnological tasks. The main project is the creation of a platform for cell analysis which shall be especially used for the study of bacterial intercommunication and for bacteria sensing, for the sake of fighting diseases without the use of antibiotics.

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Chapter 9. BIOSENSORS AND BIOREACTORS BASED ON LACCASE FOR ENVIRONMENTAL APPLICATIONS

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Abstract. *Technogenic pressure on the environment, as well as irrational unhealthy eating, has significantly affected the human health. To eliminate these negative impacts it is greatly important a creation of new highly effective approaches for analysis of the levels of water pollution, food quality and physiological human markers for clinical diagnostics. The presented work demonstrates the development of a material sciences platform for novel biosensing and biotechnology approaches. This platform is created by bringing together the advances in ion beam technologies, ion track-based electronics and organic-inorganic hybrid materials science, and forms a principally new foundation for interfacing conventional electronics and nanoelectronics with bio-active sensor devices. Biosensor for the detection of phenolic substrates using laccase immobilized onto TiO_2S modified graphite electrode and bioreactor based on chitosan beads with encapsulated bioelement (laccase-Fe_3O_4) for degradation of diclofenac are reported as an example.*

Keywords: *biosensors, bioreactors, laccase, xenobiotics, biodegradation.*

1. Biosensors

In recent years, there is a growing interest in the world in the development of new highly specific approaches of identifying the key ingredients or metabolites which determine the quality of the product or serve as markers for diseases, the physiological state of a human organism or environmental safety. Among different analytical approaches, a special role is attributed to analytical biotechnology which exploits the principles of biomolecular recognition, highly developed during the evolution. Biosensors are the most novel achievement of analytical biotechnology.

Biosensors are not only the object for basic and applied sciences, but also an important commercial product in industrially developed countries. According to the new market research report "Biosensors Market by Application (POC, Home Diagnostics, Research Labs, Biodefense, Environmental Monitoring, Food & Beverages Industry), Technology, Product (Wearable and Non-Wearable), and Geography – Global Forecast to 2022", the market is expected to be valued at USD 27.06 Billion by 2022, growing at a CAGR of 8.84% between 2017 and 2022. The market growth is driven by the continuous technological advancements in the biosensors ecosystem, increase in the use of biosensors for medical and nonmedical applications, lucrative growth in POC diagnostics, and rise in the demand for glucose and lactate monitoring systems.

Generally, a biosensor is a hybrid device containing two functional parts: a bioelement (*biorecognition unit*) – an immobilized biologically active material and a physical transducer (*signal converting unit*). The biosensor bioelement is usually prepared in the immobilized form and often covered with an outer membrane (or placed between two membranes in a sandwich manner), which either prevents the penetration of interfering substances into a sensitive bioselective layer and transducer surface, or creates a diffusion barrier for the analyte. Such membrane structures increase the stability of the biorecognizing element, enhance its selectivity and provide the diffusion limitations for biochemical reactions.

Enzymatic sensors are divided into electrochemical, optical, and thermal biosensor according to transduction methods (Fig. 1). Nowadays, nanotechnology approaches have been successfully used for improvement of functional properties of the enzymatic sensors. The integration of micro- and nanotechnologies seems to be very promising in further development and production of such biosensors due to the unique combination of chemical inertness, surface chemistry, size- and shape-dependent electrochemical and optical properties.

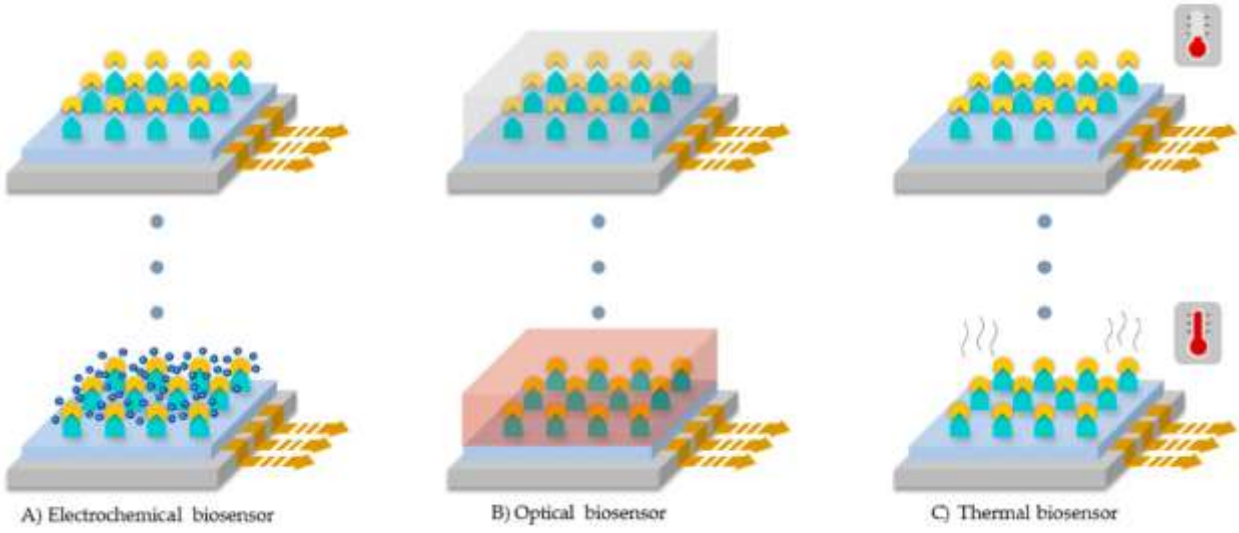


Fig. 1. Enzyme-based biosensor systems according to the transduction method used. (A) Electrochemical biosensor, (B) optical biosensor, and (C) thermal biosensor [1]

1.1. Biosensors based on laccase

Technogenic pressure on the environment significantly affects the pollution of water resources, resulting in that one of the most important problems is the contamination of soil, water, and air by toxic chemicals. Due to the extremely rapid development of the industrial sphere and wide use of plastic, detergents, pharmaceuticals, pesticides in agriculture, the level of environmental pollution is growing up extremely fast that causes a serious threat to human life and health.

Especially dangerous products of the chemical and pharmaceutical industry are xenobiotics which are classified also as carcinogens that cause disruption of the endocrine system of human and animals. They are a matter of industrial origin in the human body, capable of causing effects similar to the effects of high doses of a natural hormone estrogen [2, 3]. Mimicking estrogen, they adversely affect the function of the endocrine system and able to cause various health defects, affecting synthesis, metabolism and cellular reactions of natural estrogens [4-7].

Xenobiotics are a common pollution. They arrive in the surface water with drains of oil, shale, cox-chemical, textile, forest-chemical, and pharmaceutical industries as well as with drains of hydrolysis industry. For example, xenobiotic Bisphenol A is a monomer that is used for the manufacture of polycarbonate plastic and epoxy resins, which are raw materials for the production of packaging materials for food and drinks. World market of Bisphenol A is over 6.4 billion pounds per year, and thus, it is one of the chemicals with the highest volume of production all over the

world [8]. As a result of hydrolysis of the ester linkages in these polymers, Bisphenol A is released in the environment, resulting in widespread negative impact on human and animals.

A list of substances with an endocrine activity is constantly expanding. It includes chlorine-organic and poly-aromatic compounds, the source of which is a plastic used for packaging of drinking water [9], and some pharmaceutical drugs widely used, such as Ibuprofen, in dangerous concentrations. When using Ibuprofen in the order of hundreds of thousands of tons (Germany), the anti-inflammatory drug and its metabolites are detected in all samples of wastewater and sea water at a concentration from 0.1 to 20 μg/L [10]. The main sources of substances with xenoestrogenic effect are the wastewater of cities and animal complexes. A high content of estrogens and pharmaceutical drugs is still existed even after treatment of water [11]. Xenobiotics, classified as carcinogens, are toxic to healthy compounds that cause disruption of the endocrine system of human and animals. The development of new approaches for monitoring of these dangerous substances coming from the wastewater is a topical problem to improve human life first of all.

Recent progress in nanobiotechnology allows using micro- and nanomolecular approaches for improvement of bioanalytical parameters of the sensors – selectivity, response time, miniaturization of the biorecognition unit [12]. For example, the use of ordered mesoporous silica materials including SBA-15 attracted substantial attention in electrochemical biosensing (Fig. 2), as an efficient template (large surface area and pore size) for the synthesis of nanocomposites [13, 14]. For example, SBA-15 used as immobilizer for GOD, Cyt C, antibody [15] and also for methanol detection (Fig. 3) [16]. Also, due to high efficiency, recyclability, sustainability, and eco-friendly properties have shown to be suitable for a wide-ranging application, including pollutant sensing [17, 18], environmental remediation [19], and diagnosis of disease [20]. On the other hand, the usage of microporous carbon fibers and nanoparticles of noble metals for the construction of new highly sensitive biosensors for phenols as well as *L*-lactate analysis has been proved recently in [21-23].

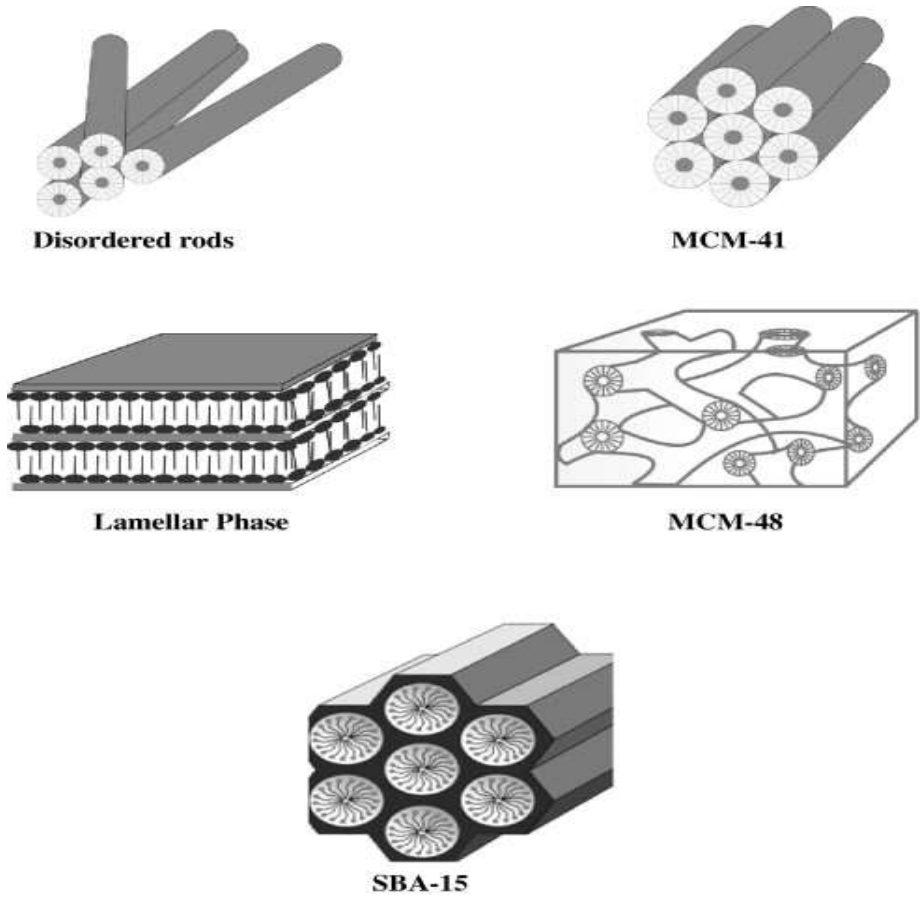


Fig. 2. Porous materials for electrochemical applications [13, 14]

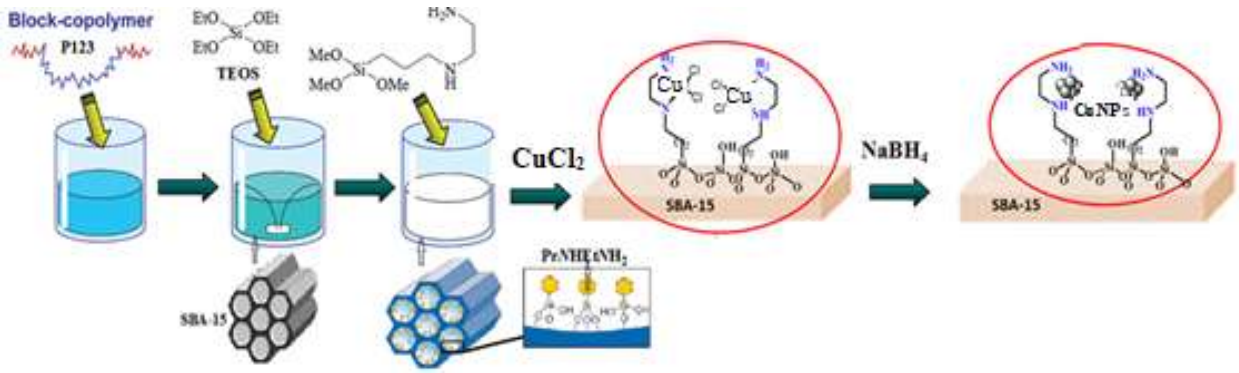


Fig. 3. Schematic figure for preparation of palladium nanoparticles supported on Santa Barbara Amorphous-15 (SBA-15) as nanocatalysis platform for methanol detection [16]

Concerning the laccase application for the targeted oxidation of phenolics, the development of biocatalysts application which are stable under environmental condition is a critical issue, which is currently addressed by many work initiatives at EU level, e.g. Knowledge Based Bio Economy (KBBE) programme. Moreover, efficiency of usage laccase form *Trametes versicolor* for effectively removal of C-bisphenol A and C-sodium diclofenac from secondary effluent from a municipal wastewater was demonstrated recently [25]. Also recently, innovative amperometric biosensors for monitoring the level of wastewater pollution have been constructed [26] on the surface of the gold planar electrodes C220AT "DropSens" by using the organic-inorganic ureasil-based composites as host polymer matrixes and immobilized commercial laccase from *Trametes versicolor*. In fact, urea-silicates (shorten ureasils) are well-known as representatives of organic-inorganic hybrid polymer materials successfully examined as dispersion media for luminescent Eu^{3+} salts [27], ion conducting Li^{+} salts [28], organic dyes [29], semiconductor and metal nanoparticles [30-33], and, for the first time, the ureasil-based composites were tested for immobilization of laccase and construction of biosensors [26]. It has been found that the biosensor based on the ureasil-chalcogenide glass composite was characterized by very high sensitivity to be 38.3 times higher in compare with pure ureasil. On the other hand, application of the ureasil-chalcogenide glass composite with incorporated silver nanoparticles synthesized by high-dose 30 keV Ag^{+} ion implantation results in decreasing the biosensor sensitivity up to 2390 times. The results obtained indicated a well expressed influence on the sensor's characteristics of the constructed biosensor by organic-inorganic ureasil-based matrixes and silver nanoparticles.

Laccase-based amperometric enzyme biosensors of the third generation for analysis of phenol derivates have also been constructed [34] using graphite rods (type RW001) as working electrodes and the photocross-linked polymers as a matrix. Such matrix consisted of epoxidized linseed oil (ELO), bisphenol A diglycidyl ether (RD) as reactive diluent and 50% mixture of triarylsulfonium hexafluorophosphate in propylene carbonate (PI) as photoinitiator. The synthesis was made by the reaction of ELO and 10 mol.% or 30 mol.% of RD, using 3 mol.% of PI (ELO/10RD and ELO/30RD, respectively). The holding matrixes were used for an immobilization of commercial laccase from the fungus *Trametes versicolor*. A correlation between the constructed biosensor parameters and microscopical free volume of the biosensor holding matrixes was established.

In this study we reported the first results to design a biosensor using sulfur-doped titanium dioxide nanoparticles (TiO_2S) and laccase for phenol analysis. Monitoring of phenolic compounds in the food industry and for environmental and biomedical analyzes using portable, cost-effective devices has become an area of growing interest over the past decade. Phenolic compounds are widespread in nature. They can be found in fruits and vegetables, and they are responsible for the organoleptic properties of some foods, such as wine and olive oil. Phenols are also the breakdown

products of natural organic compounds such as humic substances, lignins and tannins. However, some phenols are ubiquitous pollutants that enter natural waters with chemical effluents.

The TiO_2 samples were prepared by controlled hydrolysis of titanium butoxide, followed by hydrothermal treatment according to a modified method [35]. In a typical synthesis, 20 mL acetic acid was added dropwise to a flask containing 10 mL titanium butoxide, diluted in 30 mL absolute ethanol, followed by the addition of 1.5 mL H_2SO_4. The obtained clear solution was sonicated at 40 °C for 1 hour and at 60 °C for 3 hours, resulting in the formation of a sol, which was further transferred into a stainless autoclave and kept at 120 °C for 12 h. The precipitates were separated, washed thoroughly several times, dried in air at 100 °C for 24 hours, and calcined at 375 °C for 2 h. The nanoparticles sample was labeled as $TiO_2S(1.5)$, in which the number in parentheses refer to the quantity of H_2SO_4 (mL) used in the synthesis.

Laccase enzyme (E.C. 1.10.3.2) from *Trametes versicolor* with activity of ≥10 $U \cdot mg^{-1}$, 2,2′-azino-*bis*(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS), 99%, phenol, Nafion®, and other reagents and buffer compounds were purchased from Sigma-Aldrich (Darmstadt, Germany). All chemicals and reagents were prepared using triple distilled water. Prior to analysis, the raw wastewater sample was filtered through a Millipore 0.45 μm filter.

The formation of bio-nanocomposite membrane of the biosensor was performed as follows. 10 μL of enzyme solution (with a concentration of 1 $mg \cdot mL^{-1}$ and a volumetric activity of 13.6 $U \cdot mL^{-1}$, in 50 mm acetate buffer, pH 4.5) was mixed with 5 μL of a colloidal solution of TiO_2S (sulfur dopant content) particles (1 $mg \cdot mL^{-1}$) in 1 % aqueous solution of Nafion. The formed mixture was dropped onto the 3.05 mm diameter carbon rod working electrode, with the next step of drying the resulting mixture (nanoparticles + enzyme + polymer) for 10 min occurring in air at room temperature. After drying, mechanically strong TiO_2S-enzyme-Nafion membrane was formed on the surface of the carbon rod electrode. The prepared bio-nanofunctionalized electrodes were rinsed with 50 mM acetate buffer, pH 4.5 and kept at 4 °C till usage.

The amperometric biosensor was constructed in a three-electrode configuration to be used for constant-potential amperometry. The biosensor configuration included a Pt counter electrode, Ag/AgCl/KCl(3M) reference electrode, and a graphite-rod (type RW001, 3.05 mm) from Ringsdorff Werke (Bonn, Germany) as a working electrode. Before usage, the working electrodes were polished with P2000 emery paper. The amperometric analysis was carried out using a potentiostat CHI 1200A (IJ Cambria Scientific, Burry Port, UK) in an electrochemical cell at room temperature, avoiding direct light and under continuous stirring.

The cyclic voltamperometric analysis using ABTS as a model laccase substrate was performed to estimate the optimal working potential of the reduction of electroactive products of the laccase's reaction with a bioelectrode based on Nafion/TiO_2S membrane (Fig. 4). It has been shown that the addition of ABTS to the measuring cell resulted in an increase in the cathodic currents of the bio-nano-modified electrode. The significant increase in the currents clearly indicates the high efficiency of electron transfer between the electroactive product of the laccase reaction and the Nafion/TiO_2S-modified surface of the working electrode. In addition, it allows for estimation of the optimal operating potential for the biosensor, which corresponds to -150 mV *vs* Ag/AgCl/KCl(3M) reference electrode. Such relatively low operating potential makes the biosensor more selective, avoiding the interfering response of electroactive chemicals, contained in real samples, which can be auto-oxidized or auto-reduced at extreme operating potential values. That is why the potential of -150 mV *vs* Ag/AgCl was chosen as optimal for further work to study the characteristics of the designed bio-nano-modified electrodes.

The amperometric measurements were performed in a glass electrochemical cell with a working volume of 50 mL, filled with 20 mL acetate buffer, pH 4.5 at room temperature, avoiding direct light. For cyclic voltamperometry, operational conditions were as follows: scanning rate 7 $mV \cdot s^{-1}$ *vs* Ag/AgCl/KCl(3M) reference electrode in 50 mM acetate buffer, pH 4.5 at room temperature. For chronoamperometric analysis, the bio-nano-modified electrodes were placed in a vigorously stirred solution and, after setting the base signal at an operating potential of -150 mV *vs* Ag/AgCl, increasing aliquotes of laccase substrate (phenol) were added to the measuring cell.

The developed $TiO_2S(1.5)$-modified bioelectrodes were characterized toward phenol, as the most common pollutant of wastewater (Figs. 5 and 6).

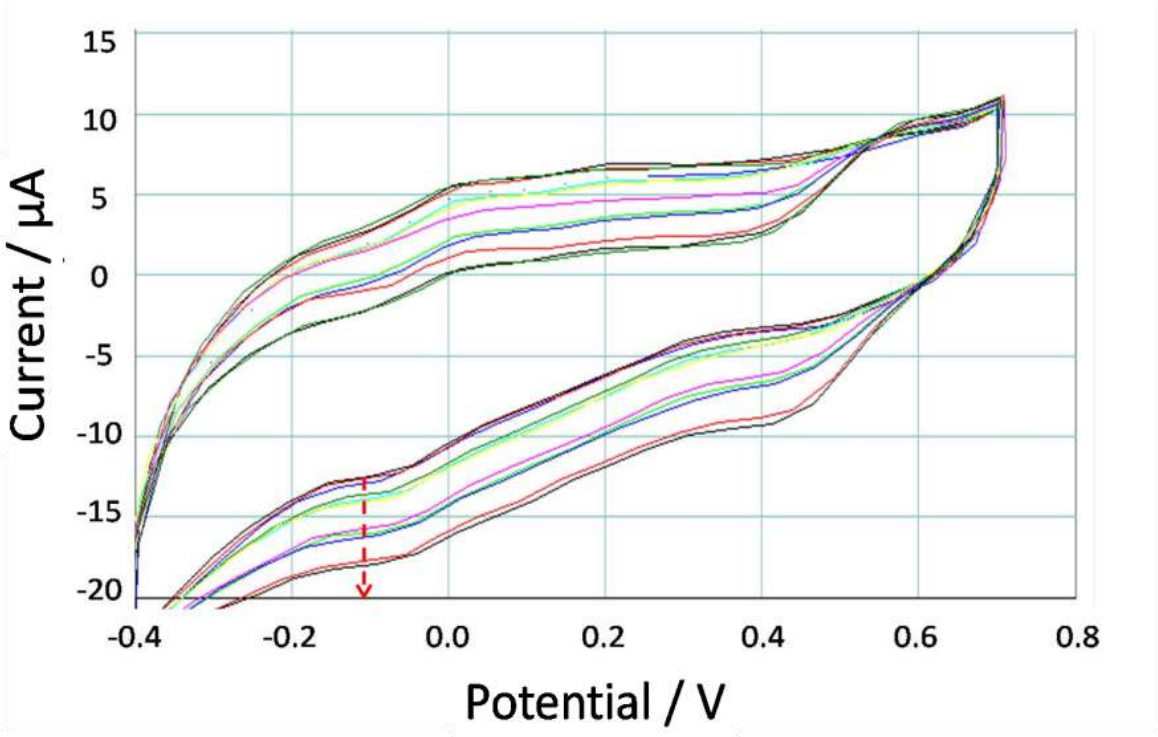


Fig. 4. Cyclic voltammogram of the bio-nano-modified electrode based on Nafion/$TiO_2S(1.5)$ composite membrane before (black line) and after addition of increasing concentrations of ABTS (color lines). Conditions: scanning rate 7 $mV \cdot s^{-1}$ vs Ag/AgCl, 50 mM acetate buffer, pH 4.5

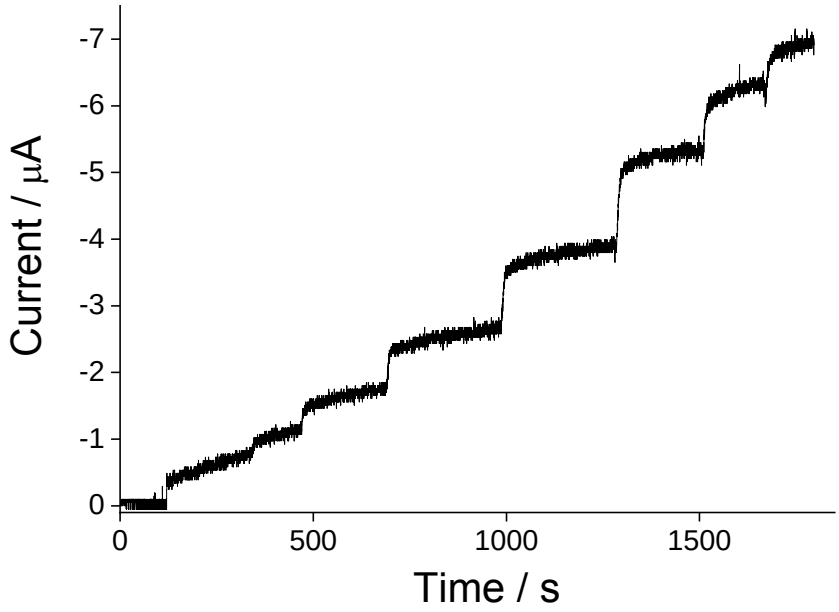


Fig. 5. Chronoamperometric response of $TiO_2S(1.5)$-modified bioelectrodes toward subsequent addition of increasing concentrations of phenol

Evaluation of the dependence of the operating parameters of Nafion/TiO_2S-modified laccase bioelectrodes was performed according to four main parameters: I_{max} – the maximal response of the biosensor at substrate saturation; K_M^{app} – the apparent Michaelis-Menten constant; linearity, and sensitivity. The $TiO_2S(1.5)$-modified bioelectrodes were characterized by an I_{max} value of 7.97±0.35

μA; K_M^{app} – 0.36±0.05 mM, the upper linearity at about 0.4 mM for the phenol, and with a sensitivity of 1168 $A \cdot M^{-1} \cdot m^{-2}$.

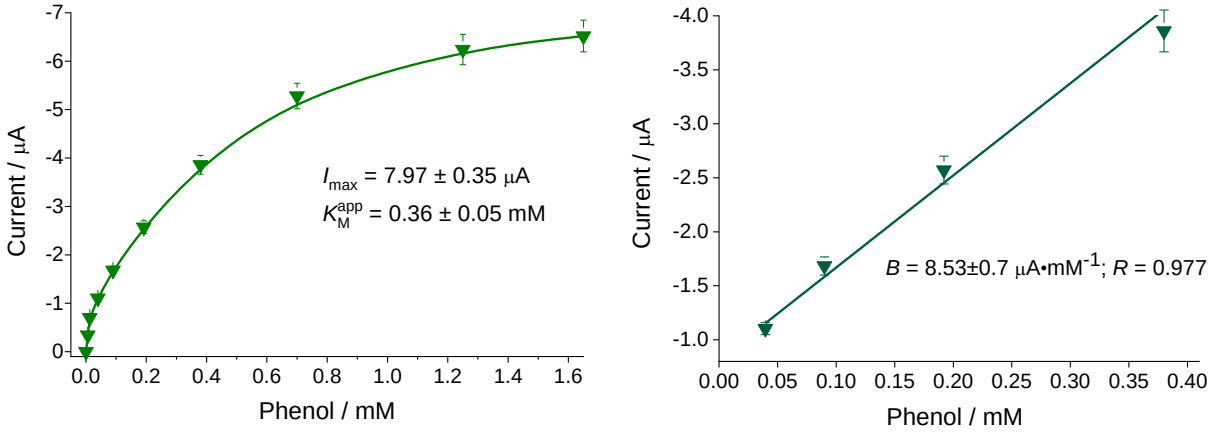


Fig. 6. Chronoamperometric calibration curves of $TiO_2S(1.5)$-modified bioelectrodes response for substrate saturation (left) and within the linear range (right) toward subsequent addition of phenol. Abbreviations: I_{max} – maximal response of the biosensor at substrate saturation; K_M^{app} – the apparent Michaelis-Menten constant; B – slope of the curve; R – correlation coefficient for the linear regression

The developed $TiO_2S(1.5)$-modified bioelectrodes were used for analysis of phenol in a communal wastewater sample that had been collected at the treatment plant of Lviv (Ukraine) prior to its purification. The wastewater samples were spiked with phenol analytes prior to the analytical experiments with the biosensor electrodes. The spiked samples were made by the addition of 1.0 mM phenol to the raw wastewater. The biosensor analysis was performed using a "standard addition mode" using different dilution steps (10 and 40) of the samples (Fig. 7).

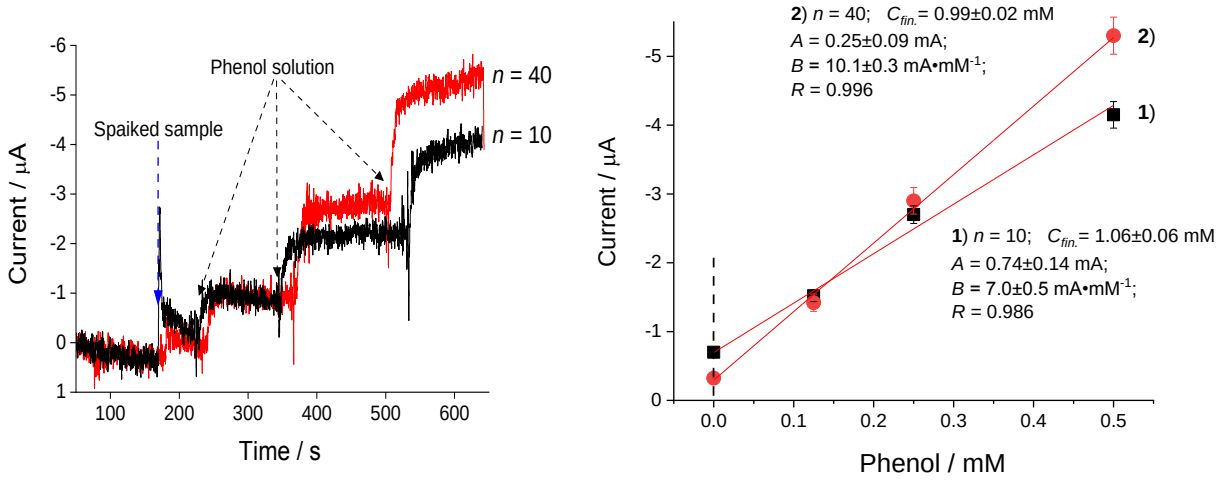


Fig. 7. The chronoamperometric response for the biosensor analysis of phenol in the spiked communal wastewater samples using a "standard addition mode". Abbreviation: n – dilution step (left); and the calibration curves for biosensor analysis of phenol in the spiked communal wastewater samples using a "standard addition mode". Abbreviations: n – dilution step; $C_{fin.}$ – estimated final phenol concentration; A – analyzed sample output; B – slope of the curve; R – correlation coefficient for the linear regression (right)

The biosensor analysis demonstrated the following results for phenol content in the diluted samples (the initial sample was spiked with 1.0 mM phenol): 0.99±0.02 mM for n = 40 dilution steps and 1.06±0.06 mM for n = 10, respectively (Fig. 7). The calculated average of the tested phenol concentration is equal 1.02±0.04 mM that corresponds to 98% of assay accuracy. Moreover,

this result demonstrates an absence of any interfering impact of the compounds of raw wastewater on biosensor analysis.

2. Bioreactors

The main strategy of biodegradation of xenobiotics by microorganisms is their transformation into products that can be used for catabolic and anabolic processes (for energy production and synthesis of cellular substances). Biodegradation and complete mineralization can be carried out using aerobic and anaerobic microorganisms. Aerobes oxidize organic compounds with redox enzymes. In turn, anaerobes decompose xenobiotics by fundamentally different mechanisms. The terminal acceptor of electrons in anaerobes is not oxygen, but nitrate, sulfate or carbon dioxide. A group of different microorganisms is more effective in destroying foreign substances than monocultures. The ability to biodestruct organic xenobiotics is inherent in both gram-positive (Nocardia spp., Mycobacterium spp., Corynebacterium spp., Arthrobacter spp., Bacillus spp.) and gram-negative (Pseudomonas spp., Acinetobacter group, Cynetobacter spge. Xanthomonas spp.) organisms and fungi [36, 37].

Most xenobiotics are insoluble in the aqueous phase, so it is difficult for prokaryotes to transport them into the cell because they are insoluble in the soil. Bacteria have developed special mechanisms for this. The cell synthesizes rhamnolipids, which are exometabolites. According to their physicochemical properties, they are amphiphilic compounds, ie one part of the rhamnose molecule is hydrophilic, and the lipid part of the molecule is hydrophobic. These molecules form micelles, which contain hydrophobic organic compounds and in this form penetrate into prokaryotic cells. Industrial production of biocatalysts based on technical enzymes is economically viable. Various enzymes are widely used in industrial biocatalytic processes; of particular interest are laccase [38]. The broad substrate specificity of these enzymes and their ability to use atmospheric oxygen as an electron acceptor makes laccase a promising component of various industrial processes – bleaching and decolorization of tissues, delignification of pulp, production of antibiotics and anticancer drugs, wastewater treatment from organic pollutants [39]. The study of biocatalytic alternative methods of wastewater treatment from pollutants today helps to reduce the impact of toxic substances on the environment [40] and is relevant because in Ukraine, unfortunately, we have a very difficult environmental situation.

2.1. Bioreactors based on laccase

Strains of microorganisms were grown in a mineral medium of the following composition (g/l): KNO_3 – 2.5 g, KH_2PO_4 – 2 g, NaCl – 0.5 g, $MgSO_4 \cdot 7H_2O$ – 0.5 g, mineral elements – 2 ml, yeast extract – 0.2 g, which was dissolved in distilled water. The source of carbon, depending on the purpose of the experiment were: glycerol – g, glucose – 10 g, maltose – 10 g. Strains were grown at 20-25 °C or 28 °C in 500 ml flasks on a shaker with constant aeration (150 and 200 rpm). Laccase *Trametes zonate* was obtained from the culture fluid. To do this, up to 80% of ammonium sulfate was added to 100 ml of extracellular laccase culture fluid, kept cold and unscrewed. The obtained laccase preparation was used to construct a bioreactor.

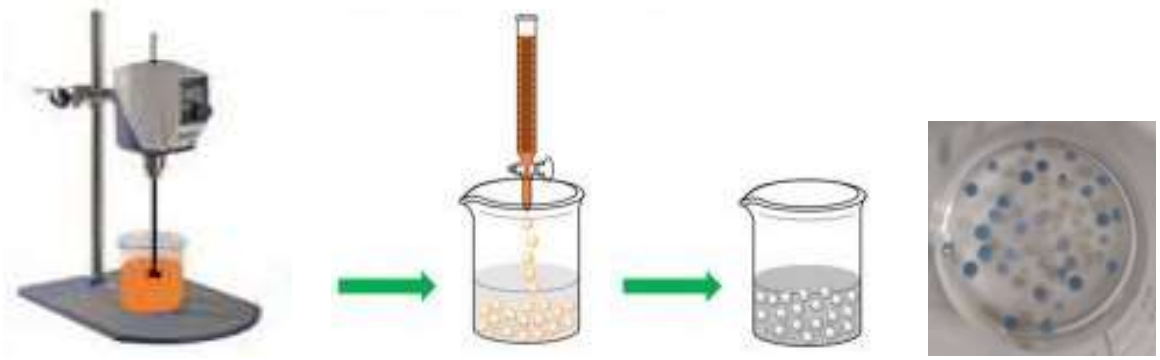
For the construction of the bioreactor, laccase was used as a degrading element due to its wide range of substrates, the ability to oxidize organic and some inorganic substrates, as well as the ability to remove xenobiotics from industrial effluents and change the color of dyes. In addition, compared to the peroxidase enzyme, the laccase enzyme does not require hydrogen peroxide (H_2O_2), but can oxidize organic compounds with oxygen (O_2) as a substrate [38].

To build a laboratory prototype of the bioreactor, strong chitosan beads with encapsulated laccase and magnetic nanoparticles (Fe_3O_4-NPs) were used. Used magnetic nanoparticles (MNPs), had the advantage due to their properties of magnetism, it is possible to control their movement and accumulation at a distance. Chitosan was chosen for the formation of balls due to its high sorption properties to organic compounds and mechanical stability.

A solution of chitosan (0.5 ml) with a concentration of 20 g/l was taken mixed with 0.05 ml of laccase solution (1 mg/ml) and 0.1 ml of suspension pre-treated with ultrasonic Fe_3O_4-NPs (30 mg/ml), stirred vigorously for 15 minutes at room temperature. The crosslinking agent was

glutaraldehyde (GA). Therefore, the resulting mixture was collected in a volume of 0.01 ml and added dropwise to 0.05 M solution of GA, which was kept for 0.5 h to form beads, then washed and used for analysis.

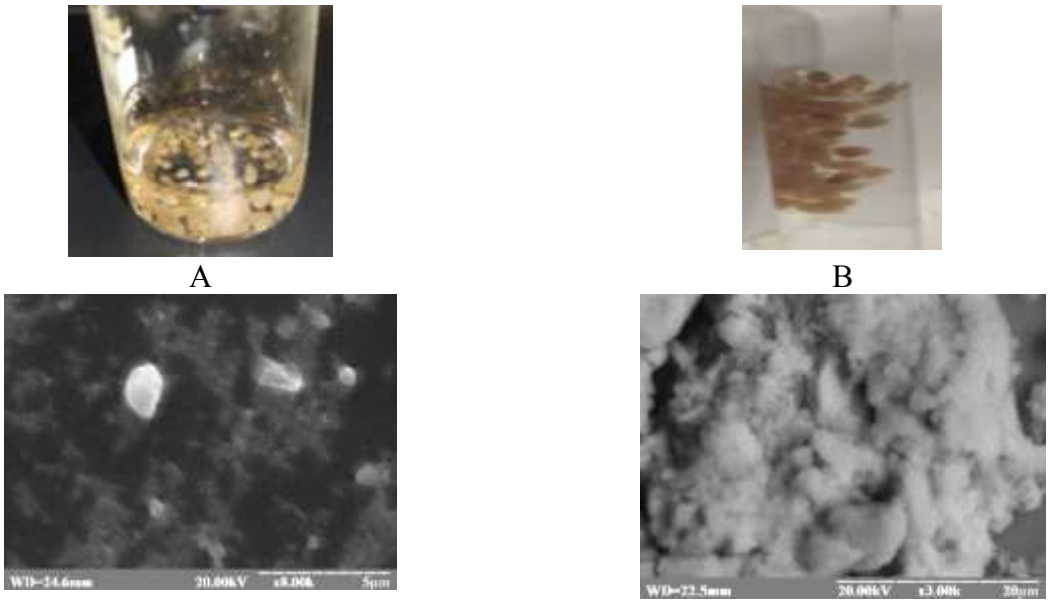
The schematic diagram of the formation of chitosan beads with encapsulated enzyme and MNPs is presented in Fig. 8.



Chitosan + MNPs + laccase 0.05 M solution of GA Laccase-Fe_3O_4-NPs-chitosan

Fig. 8. Schematic diagram of the formation of chitosan beads with encapsulated bioelement: laccase-Fe_3O_4- NPs

Photo of the appearance of the formed chitosan balls with a bioelement: laccase-Fe_3O_4-NPs is presented in Fig. 9.



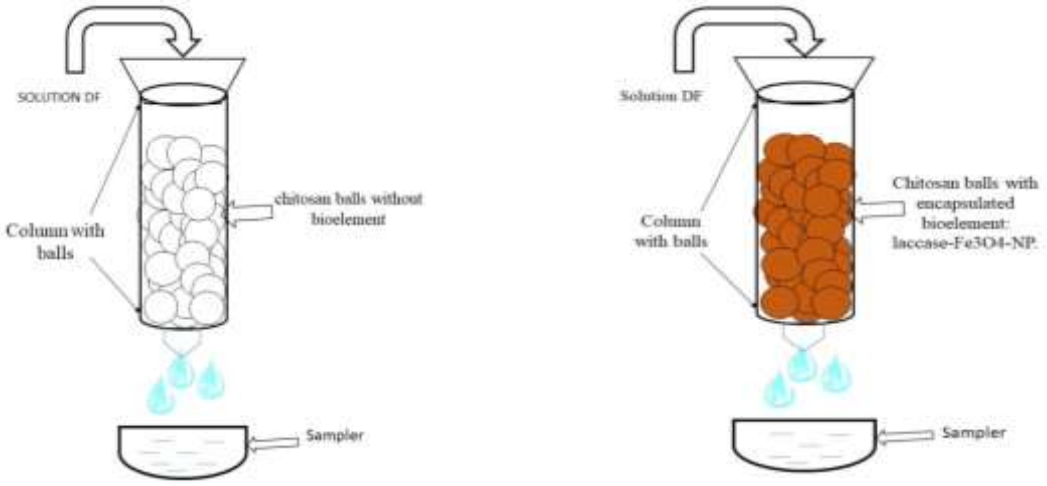
A B

C D

Fig. 9. Photo of chitosan beads with encapsulated bioelement: laccase-Fe_3O_4-NPs. A – encapsulated with laccase-Fe_3O_4-NPs in buffer; B – test tube near the magnet with encapsulated laccase-Fe_3O_4-NPs; SEM images: C – laccase-Fe_3O_4-NPs; D – encapsulated laccase-Fe_3O_4-NPs

The obtained laccase/chitosan beads were packed in column-type tubes and used as a bioreactor in experiments for bioremediation of diclofenac (DF) in model solutions (Fig. 10). The bioreactor with chitosan beads without laccase and MNC served as a control. Model solutions of diclofenac at a concentration of 0.05 mm were slowly passed (with a flow rate of 0.005 ml/(min·cm^2)) through column-type bioreactors filled with beads with and without a bioelement. Then in the resulting solutions that passed through the column the content of diclofenac was

analyzed. DF concentrations during degradation in the bioreactor were measured by HPLC (Dionex UltiMate 3000) with an ultraviolet detector (Dionex UltiMate 3000 RS Variable wavelength detector) using a moving phase of 2 mM acetic acid: methanol (30:70 rpm), UV detected at 274 nm.



A B

Fig. 10. Photo of column type laboratory bioreactor with chitosan beads with and without bioelement. A – control column without laccase-Fe_3O_4-NPs; B – experimental column with an encapsulated element of laccase-Fe_3O_4-NPs

The dynamics of DF bioremediation in the bioreactor was studied in both variants: from the control column and from the column with laccase and MNPs (Fig. 11). In the solutions obtained from the control column there was no complete oxidation of DF, while in the solutions from the experimental column the concentration of DF decreased 3 times and was 0.015 mM (Fig. 11). It was shown that the designed column had the ability to neutralize phenol for 2 weeks, with daily (1 time per day) use. The obtained results emphasize the importance of laccase in its ability to oxidize phenolic compounds. Designed bioreactors can be used to treat pharmaceutical industrial effluents.

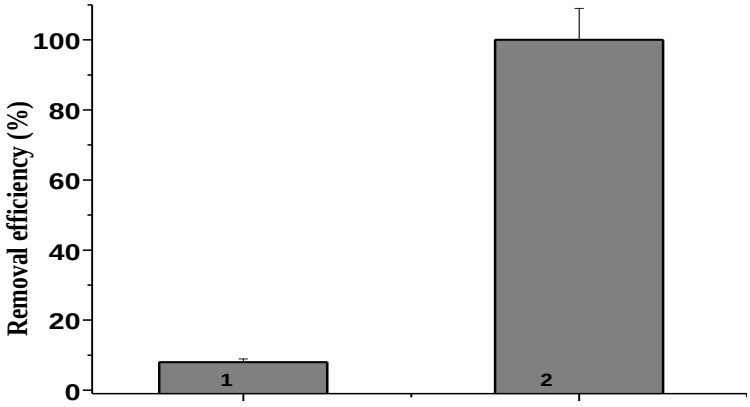


Fig. 11. Biodegradation of DF in laccase bioreactor. 1 – experimental column with encapsulated element laccase-Fe_3O_4-NPs; 2 – control column without laccase-Fe_3O_4-NPs

Conclusion

The biosensor based on laccase and TiO_2S modified graphite electrode is developed. The Nafion/TiO_2S-laccase bioelectrodes are characterized by I_{max} = 7.97±0.35 μA, K_M^{app} = 0.36±0.05 mM, the upper linearity at about 0.4 mM for the phenol, and with a sensitivity of 1168 $A \cdot M^{-1} \cdot m^{-2}$.

The developed TiO_2S-based bioelectrodes were tested for phenol analysis in the real communal wastewater sample spiked with this analyte and demonstrated a high accuracy of the analysis. A laboratory prototype of a bioreactor based on chitosan beads with encapsulated bioelement: laccase-Fe_3O_4-NPs for biodegradation of xenobiotics (DF) in model solutions, was successfully constructed.

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Chapter 10. THE INFLUENCE OF COMPLEX FERTILIZERS ON BIOLOGICAL AND MORPHOLOGICAL INDICES OF MEDICINAL PLANTS (*DESMODIUM CANADENSE AND MENTHA PIPERITA),* CULTIVATED IN THE CONDITIONS OF PRECARPATHIAN ZONE

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Abstract. *The article considers the results of researches carried out regarding the influence of complex fertilizers (Nitroamofoska, Ecolyst and Stymovit Ferti) on morphological indices, yield capacity and content of photosynthetic pigments in the leaves of medicinal plants (Desmodium canadense and Mentha piperita) in the conditions of Precarpathian area of Ukraine.*

We have established by the results of researches, that complex mineral and organic fertilizers (Nitroamofoska, Ecolyst and Stymovit Ferti) had some influence on morphological indices, yield capacity and content of photosynthetic pigments in the leaves of medicinal plants (Desmodium canadense and Mentha piperita), cultivated in soil and climatic conditions of Precarpathian area of Ukraine. The best indices we have received in the variant, where applied Nitroamofoska during pre-sowing cultivation within the norm $N_{30}P_{30}K_{30}$ and double applying fertilizer Ecolyst (the 1st time – in the plant regrowth phase 5 l/ha and the 2nd time – in the budding phase 5l/ha).

Keywords: *complex fertilizers, Nitroamofoska, Ecolyst, Stymovit Ferti, medicinal plants, Desmodium canadense, Mentha piperita, morphological indices, yield capacity, photosynthetic pigments, soil and climatic conditions of Precarpathian region.*

Introduction

The intensive search for natural medicines is observed for the last years for treatment and preventive measures against different human diseases. Hereby, the special attention is paid regarding medicinal plants as a source of biological active substances.

To provide high quantitative and qualitative indices of medicinal plant raw material more and more species of medicinal plants in Ukraine are being cultivated in the culture.

Desmodium canadence is a new and interesting plant for the flora of Ukraine. This is an ecologically plastic species and practically is not vulnerable to diseases and pests. It is considered to be perspective for cultivation throughout the whole territory of Ukraine as a perennial beans fodder and medicinal plant. In a green mass of *Desmodium canadence* in the period of flowering there are: around 4% protein, 7.6% extractive substances, 0.8% sugars, to 61 mg/100g vitamin C, 5.3 mg/100 g carotene [1].

The complexity of cultivation of medicinal plants in the world in the whole and in Ukraine particularly, is connected with their special requirements to the conditions of cultivation.

Working out and improvement of existing technologies with applying new agrarian measures do not allow not only to reduce the cost of cultivation of raw plant material due to innovations, but to increase considerably both yield capacity and quality of raw material. An important reserve of increasing yield capacity of medicinal plants and improvement of the quality of plant pharmaceutical raw material is the usage of fertilizers, namely, complex organic and mineral humic

ones. They are more often becoming the integral elements of cultivation technology of different cultures. That is why the study of influence of norms, terms and ways of complex mineral and organic humic fertilizers on morphological indices and yield capacity of medicinal plants (*D. canadense* and *M. piperita)* in the conditions of the West Ukraine, particularly, it is rather actual in the conditions of Precarpathian region.

Analysis of recent publications

Complex mineral fertilizers of the new generation are currently rather actual with balanced ratio of macro- and microelements, which were made with up-to-date technologies and contain easily digestible nutrients in the form of chelates EDTA in the complex with organic acids, that practically are totally absorbed by plants. One of such new complex mineral fertilizers is Ecolyst (company EKOPLON, Poland) [7].

The results of scientific researches have shown that microelements in the form of chelates are the most available for agricultural plants. In studies on wheat plants, treated with modern complex fertilizers, the increase of yield capacity and grain quality. The effect on the net productivity of photosynthesis and yield capacity of winter wheat [3, 5, 6].

For the last years a lot of scientists paid an attention to the peculiarities and needs of agricultural plants in microelements, particularly the effectiveness of the usage of chelated elements on the productivity of agricultural plants.

The researches carried out in the conditions of Precarpathian region also have shown that, complex mineral fertilizers Ecolyst, Rozasol and Nutrivant Plus have a positive impact on the growth of oil radish, improve morphological indices (increase the stem height and including inflorescences, the number of pods on one plant) and increase yield capacity of grain. Applying the complex mineral fertilizer Ecolyst in two terms: in the germination and rosette phase within the norm on 4,5 l/ha, yield capacity of oil radish grain increased by 5,1 cwt/ha and was 16,3 cwt/ha. The complex mineral fertilizer Nutrivant Plus increased yield capacity of oil radish grain by 4,5 cwt/ha compared with the control variant of the research [2]. However, there are almost absent researches in which the study of the influence of chelated liquid fertilizers on the productivity of medicinal plants would carry out.

The purpose of the researches

The purpose of our researches was to study the influence of norms and terms applying complex fertilizers (Nitroamofoska, Ecolyst and Stymovit Ferti) on the morphological indices, yield capacity and content of photosynthetic pigments in the leaves of medicinal plants (*D. canadense* and *M. piperita).*

Material and methods of the researches

The researches were carried out in the field crop rotation on the educational and experimental plot of Drohobych Ivan Fanko State Pedagogical University during 2020. The soils of the field, on which we carried out the researches were sod-podzol medium loamy ones with the following agrochemical indices: content of humus in the arable layer was – 2.08 %, nitrogen (by Tiurin-Konova) – 84 mg/kg soil, phosphorus (by Kirsanov) – 147 mg/kg soil, boron – 0.98 mg/kg soil, manganese – 12.4 mg/kg soil, copper – 1.44 mg/kg soil, zinc – 4.02 mg/kg soil, the reaction of soil solution is weakly acidic (pH salt – 5.8).

What concerns meteorological conditions in 2020, they were somehow different from the average long-term ones, especially, during the vegetation of plants. In this period the average monthly temperature differed slightly from the long-term average one. Hot and dry weather in May-June influenced on the growth and development of medicinal plants on the crop formation. However, temperature regime in August-September was rather favorable for flowering medicinal plants (*D. canadense* and *M. piperita),* an average daily temperature was in average – 19.6 ºC.

In general, the climatic conditions of Precarpathian region in 2020 were favorable for cultivation medicinal plants (*D. canadense* and *M. piperita).*

There repetition of the experiment was thrice. Accounting area of the plot – 15 m^2. The researches were carried out in accordance with the methodology of performing field experiments on the study of the main methods of growing crops [4].

For carrying out the researches we put two experiments by the following scheme:

No. variants	Complex fertilizers	Norms and terms of applying fertilizers		
		Applying fertilizers under pre-sowing cultivation (kg/ha active substance)	Foliar fertilization	
			Spraying plants in the germination phase (on 1 ha)	Spraying plants in the budding phase (on 1 ha)
1	Control (without fertilizers)	-	-	-
2	Ecolyst	-	5 l	5 l
3	Nitroamofoska + Ecolyst	($N_{30}P_{30}K_{30}$) -	- 5 l	- 5 l
4	Stymovit Ferti	-	5 l	5 l
5	Nitroamofoska + Stymovit Ferti	($N_{30}P_{30}K_{30}$) -	- 5 l	- 5 l

Study of the influence of terms and methods of application of complex mineral and organic humic fertilizers (Nitroamofoska, Ecolyst and Stymovit Ferti) on the morphological indices, yield capacity and the content of photosynthetic pigments in the leaves of medicinal plants (*D. canadense* and *M. piperita)* was carried out by putting field experiments, biometric and laboratory tests in accordance with generally accepted methods.

The biometric analysis of plant samples: linear dimensions of plants and their morphological structure were carried out by the method of metric measurement of plant height, length of inflorescences, determination the average number of stems on one plant, branching intensity (the average number of sprouts on one plant).

Herb yield accounting (*D. canadense* and *M. piperita)* was performed subdivided, that was weighing the plants from each plot separately (cut at the height 5-6 cm) whereupon the weight was recalculated on 1 hectare.

Mathematical data processing was performed by method of variation statistics of B.A. Dospehova in 1985 with the usage of computer Pentium IV (according to the program developed by the Department of Plant Technology of Lviv National Agrarian University) [4].

Complex mineral fertilizer Nitroamofoska within the norn ($N_{30}P_{30}K_{30}$) was applied under spring cultivation, and complex mineral fertilizer Ecolyst and the organic one Stymovit Ferti in the form of a solution with a knapsack sprayer (working solution was 300 l/ha), both fertilizers applied manually according to the scheme.

All the complex fertilizers have the number of advantages:

- in one granule of fertilizer there are more than two elements of mineral nutrition, that provides their high availability for the plants;
- complex fertilizers contain water-soluble readily available compounds of the elements of mineral nutrition for the plants;
- available in different brands with a wide range of usage on all types of soils and to provide physiological peculiarities of different agricultural crops;
- complex fertilizers provide stable yield capacity, high quality and environment friendliness of products.

Nitroamofoska ($N_{16}P_{16}K_{16}$) is a complex high-performance concentrated nitrogen-phosphorus-potassium granular fertilizer, which is produced in various brands with different

content and ratio of mineral nutrients: N:P:K = 16:16:16 and others. The main elements of mineral nutrition of plants are in water-soluble and easily accessible forms for the plants.

Phosphorus Nitroamofoska in the soil is more mobile than phosphorus superphosphate and is easily absorbed by plants. Each granule of fertilizer contains the same amount of nitrogen, phosphorus and potassium. In addition, all nutrients are evenly distributed in the soil compared to the mixed composites [9].

Complex fertilizer Ecolyst – universal multicomponent fertilizer for foliar fertilization of all cultivated crops, made by modern technologies, containing easily digestible trace elements in the form of EDTA chelates. Chelates are complex organic compounds that are easily soluble in water and provide high availability of microelements to plants in combination with organic acids that are almost completely absorbed by plants. With the help of foliar fertilizers Ecolyst can quickly improve plant under a sudden deterioration of weather conditions, during severe and prolonged stress, when growth processes are pulled back and the ability of the roots to absorb nutrients deteriorates. Applying fertilizer also allows to solve the problem of macro- and microelements lack at the first symptoms of deficiency. Fertilizer Ecolyst strengthens plants, increasing their resistance to pests and diseases.

Fertilizer Ecolyst can be used with other fertilizers, growth regulators and pesticides.

During the budding period the best effect will be given by the use of boron fertilizer Ecolyst Mono Bor (1-2 l/ha) and complex fertilizer Ecolyst Standard (4-5 l/ha). For the first feeding (for the formation of 3-6 leaves) it is recommended to make Ecolist Macro 6-12-7 (3-5 l/ha), which contains an increased amount of phosphorus [8].

Stymovit Ferti is a highly effective concentrated ecologically clean organic fertilizer made on the basis of biohumus extract. Enriched with macro- and microelements and a complex of biologically active substances of natural origin. Due to its special composition, Stimovit Ferti fully meets the needs of crops in organic and mineral nutrients.

Function: root and foliar fertilization of crops during the active growing season for the even development, stable immunity, increased ovary formation and improved quantitative and qualitative characteristics of the crop.

Composite: humic substances up to 1.5 %, N – 2.8 %, P – 2.8 %, K – 2.8 %, Ca – 0.5 %, Mg – 0.3 % and Mn, Cu, Zn, Co.

Medicinal plants (*D. canadense and M. piperita)* placed after spring wheat. After collecting the predecessor and clearing the area from crop residues, stubble was peeled to a depth of 8-12 cm with disc tools. Helios herbicide at the rate of 4 l/ha was applied three weeks before plowing in order to destroy perennial weeds.

Plowing was carried out to a depth of 20-22 cm in the first decade of October. In spring, soil leveling and pre-sowing cultivation (to a depth of 6-8 cm) were carried out.

Desmodium canadense was sown in the third decade of April. Seeds were sown with a hand drill with a disk opener of a vegetable seeder type CO – 4.2. Sowed in a row with a row spacing of 45 cm, the depth of seed wrapping – 2-3 cm, the seeding rate was (15 kg/ha). Mentha piperita was planted with rhizomes, width was – 50 cm.

Caring for medicinal plants consisted of loosening the rows and destroying weeds. During the growing season we carried out three inter-row treatments and weeding of plants.

Complex fertilizers were applied according to the experimental scheme.

Determination of the content of photosynthetic pigments in the leaves of medicinal plants Desmodium canadense and Mentha piperita

The content of chlorophyll *a, b* and carotenoids in the leaves of *D. canadense* and *M. piperita* was determined in the total extract of pigments without prior separation [10].

A portion of plant material 50 mg was ground with scissors and placed in a porcelain mortar. At the tip of the scalpel added a small amount of $CaCO_3$ (to neutralize the acids of cell juice to prevent pheophytinization of pigments), quartz sand, add 2-3 ml of 80% acetone and grind thoroughly.

The resulting extract was poured into a measuring tube of 10 ml. The mortar was rinsed several times with small portions of acetone and the volume in a test tube was adjusted to the mark with pure acetone. Then centrifuged acetone extract for 10 min at 3000 vol.

The optical density of the extract was determined at wavelengths corresponding to the absorption maxima of chlorophyll *a* and *b*, for 80 % acetone – 663 and 646 nm, respectively, and for the sum of carotenoids – at 470 nm. The solvent was used as a control.

The concentration of pigments was calculated according to Lichtentaler:

$C_{chl.a,\ mg/l} = 12.21 \cdot D_{663} - 2.81 \cdot D_{646}$,

$C_{chl.b,\ mg/l} = 20.13 \cdot D_{646} - 5.03 \cdot D_{663}$,

$C_{car.,\ mg/l} = \frac{1000 \cdot D470 - 3.27 \cdot Cchl.a - 100 \cdot Cchl.b}{229}$.

The content of pigments (A) in a vegetable raw material was calculated by the following formula:

$A,\ mg/g = \frac{C \cdot V}{S \cdot 1000}$,

where C – concentration of pigments, mg/l,

V – the volume of the extract, ml (10 ml),

S – sample of plant material, g.

Results of the research and their discussion

An important reserve for increasing the yield of medicinal plants and improving the quality of plant pharmaceutical raw materials is the use of complex fertilizers that contain easily digestible macro- and micronutrients in the form of EDTA chelates in combination with organic acids and concentrated organo-mineral humic fertilizers. They are increasingly becoming an integral part of the technology of growing different crops.

According to our research carried out in 2020, complex concentrated fertilizers had some effect on the morphological indices of medicinal plants *D. canadense* and *M. piperita*.

The best these indices were found in the variants with application of Nitroamofoska ($N_{30}P_{30}K_{30}$) under pre-sowing cultivation and subsequent application of Ekolyst and Stymovit Ferti fertilizers in two terms: the first time – when spraying plants in the germination phase (regrowth) and the second time – when spraying plants in the budding phase.

As a result of research on medicinal plants *D. canadense*, the highest morphological indices were established on the variant with applying Nitroamofoska ($N_{30}P_{30}K_{30}$) under pre-sowing cultivation and subsequent application of Ekolyst fertilizer in two terms (the first time – when spraying plants in the germination phase 5 l/ha and the second time – when spraying plants at the beginning of budding 5 l/ha). In this variant, the plant height was 82 cm. The lowest plant height (71 cm) was found in *D. canadense* plants in the control variant (without fertilizers).

As for the thickness of the stem and the length of the apical inflorescence of the plant, these figures were the highest in the variants with the introduction of Nitroamofoska ($N_{30}P_{30}K_{30}$) for pre-sowing cultivation and subsequent application of Ekolyst and Stymovit Ferti fertilizers in two terms (the first time – when spraying plants in the germination phase and the second time – when spraying plants at the beginning of budding).

These values were slightly lower in the variants when applying complex fertilizers Ecolyst and Stymovit Ferti without the application of Nitroamofoska (Table 1).

When using complex fertilizers on crops of medicinal plants *M. piperita*, the morphological indices were the highest in the variants with applying Nitroamofoska ($N_{30}P_{30}K_{30}$) under pre-sowing cultivation and subsequent application of fertilizers Ecolyst and Stymovit Ferti in two terms (the first time – when spraying plants in the regrowth phase and the second time – when spraying plants at the beginning of budding).

The highest plant height – 58 cm with a stem thickness of 9 mm was determined in the variant with applying Nitroamofoska ($N_{30}P_{30}K_{30}$) under pre-sowing cultivation and subsequent application of Ecolyst fertilizer in two terms (the first time – when spraying plants in the regrowth phase 5 l/ha and the second time – when spraying plants in the budding phase 5 l/ha).

Table 1

Influence of complex fertilizers on morphological indices of medicinal plants
Desmodium canadense

Complex fertilizers	Norms and terms of applying fertilizers			Height of the plant, cm	Thickness of stem, cm	The length of the apical inflorescence, cm
	Application of fertilizers for pre-sowing cultivation (kg/ha active substance)	Spraying plants in the germination phase (on 1 ha)	Spraying plants in the budding phase (on 1 ha)			
1. Control (without fertilizers)	-	-	-	71	0.3	22
2. Ecolyst	-	3 l	3 l	77	0.4	25
3.Nitroamofoska + Ecolyst	($N_{30}P_{30}K_{30}$)	3 l	3 l	82	0.7	28
4.Stymovit Ferti	-	5 l	5 l	76	0.3	24
5.Nitroamofoska + Stymovit Ferti	($N_{30}P_{30}K_{30}$)	5 l	5 l	79	0.6	26

Somehow lower indices were found in the variant with applying Nitroamofoska ($N_{30}P_{30}K_{30}$) under pre-sowing cultivation and subsequent applying Stymovit Ferti fertilizer. In the control variant (without fertilizers) the average height of the plant was the smallest and was only 51 cm and a stem thickness of 6 mm (Table 2).

Table 2

Influence of complex fertilizers on morphological indices of medicinal plants
Mentha piperita

Complex fertilizers	Norms and terms of applying fertilizers			Height of the plant, cm	Thickness of stem, mm
	Application of fertilizers for pre-sowing cultivation (kg/ha active substance)	Spraying plants in the germination phase (on 1 ha)	Spraying plants in the budding phase (on 1 ha)		
1. Control - (without fertilizers)	-	-	-	51	6
2. Ecolyst	-	3 l	3 l	55	8
3.Nitroamofoska + Ecolyst	($N_{30}P_{30}K_{30}$) -	- 3 l	- 3 l	58	9
4. Stymovit Ferti	-	5 l	5 l	54	7
5.Nitroamofoska + Stymovit Ferti	($N_{30}P_{30}K_{30}$) -	- 5 l	- 5 l	56	8

With regard to the effect of complex fertilizers on the formation of the number of branches on the central shoot and the diameter of the bush of medicinal plants *M. piperita*, these indices were the highest in the variant with applying Nitroamofoska ($N_{30}P_3K_{30}$) for pre-sowing cultivation and subsequent applying fertilizer Ecolyst in two terms (the first time – when spraying plants in the regrowth phase 5 l/ha and the second time – when spraying plants at the beginning of budding 5 l/ha), which were, respectively – 10 branches on the central shoot plants and a diameter of a bush of 56 cm.

These values were slightly lower in the variants without the applying of Nitroamofoska ($N_{30}P_{30}K_{30}$) for pre-sowing cultivation and subsequent applying fertilizers Ecolyst Standard and Stymovit Ferti in two terms (the first time – when spraying plants in the regrowth phase and the second time – when spraying plants at the beginning of budding).

In the control variant (without fertilizers), these indices were the lowest and amounted to – respectively, 8 pcs. branches on the central shoot and a bush diameter of 49 cm (Table 3).

Table 3

Influence of complex fertilizers on morphological indices of medicinal plants *Mentha piperita*

Complex fertilizers	**Norms and terms of applying fertilizers**			**The number of branches on the central shoot of the plant, pcs.**	**The diameter of the bush, cm**
	Application of fertilizers for pre-sowing cultivation (kg/ha active substance)	**Spraying plants in the germination phase (on 1 ha)**	**Spraying plants in the budding phase (on 1 ha)**		
1. Control (without fertilizers)	-	-	-	51	6
2. Ecolyst	-	3 l	3 l	55	8
3.Nitroamofoska + Ecolyst	($N_{30}P_{30}K_{30}$) -	- 3 l	- 3 l	58	9
4. Stymovit Ferti	-	5 l	5 l	54	7
5.Nitroamofoska + Stymovit Ferti	($N_{30}P_{30}K_{30}$) -	- 5 l	- 5 l	56	8

To form a high yield of terrestrial mass of medicinal plants requires a significant amount of moisture and nutrients. During the year of researches, the weather conditions developed in such a way that during the growing season only one mowing was carried out on the variants. In a dry year, lack of moisture and high daytime temperatures during the spring-summer period of plant growth and development slowed down growth processes. Nutrients were poorly absorbed, regrowth was slowed down, plants were poorly bushed, shoots and leaves were not formed very much. All this did not allow to form a full crop of dry raw materials and green mass after the first mowing. But even in such conditions, due to fertilizers, the yield of the studied medicinal plants (*D. canadense* and *M. piperita*) was high.

Complex fertilizers Nitroamofoska, Ekolyst and Stymovit Ferti had a significant impact not only on the morphological characteristics of plants but also on the yield of the aboveground part of medicinal plants.

As can be seen from Table 4, the yield of dry raw material (herb) *D. canadense* also depends on the effect of complex fertilizers Nitroamofoska, Ecolyst and Stymovit Ferti. The highest yield of dry raw material (herb) *D. canadense* was set in the variant with the applying Nitroamofoska ($N_{30}P_{30}K_{30}$) under pre-sowing cultivation and subsequent applying fertilizer Ecolyst in two terms (for the first time – at yield of dry raw material (herb) *D. canadense* was set on spraying plants in the regrowth phase 5 l / ha and the second time – when spraying plants at the beginning of budding 5 l/ha), which was 26.1 cwt/ha or was higher than in the control variant (without fertilizers) at 4.5 cwt/ha.

As for the yield capacity of other variants, where Stymovit Ferti complex organic fertilizer was applied, it was slightly lower than in the variant with applying complex fertilizer Ecolyst.

The lowest yields capacity was in the control variant (without applying fertilizer) that was 21.6 cwt/ha.

Table 4

Yield capacity of dry raw material (herb) of the medicinal plant *Desmodium canadense* depending on the impact of complex fertilizers

Complex fertilizers	Norms and terms of applying fertilizers			Yield capacity of dry raw material (herb)	
	Application of fertilizers for pre-sowing cultivation (kg/ha active substance)	Spraying plants in the germination phase (on 1 ha)	Spraying plants in the budding phase (on 1 ha)	cwt/ha	+; - to control
1. Control (without fertilizers)	-	-	-	21.6	-
2. Ecolyst	-	5 l	5 l	24.4	+ 2.8
3.Nitroamofoska + Ecolyst	$N_{30}P_{30}K_{30)}$ -	- 5 l	- 5 l	26.1	+4.5
4. Stymovit Ferti	-	5 l	5 l	23.5	+1.9
5.Nitroamofoska + Stymovit Ferti	$(N_{30}P_{30}K_{30)}$ -	- 5 l	- 5 l	25.2	+3.6
LRD (the least real difference) 0.5				1.8 cwt/ha	

The formation of the yield capacity of dry raw material (leaves) of the medicinal plant *M. piperita* also depended on both climatic conditions and the impact of complex fertilizers (Table 5). The highest yield capacity of dry raw material (leaves) of the medicinal plant *M. piperita* was set in the variant with applying Nitroamofoska ($N_{30}P_{30}K_{30}$) under pre-sowing cultivation and application of Ekolyst fertilizer in two terms (the first time – when spraying plants inthe regrowth phase 5 l/ha and the second time – when spraying plants at the beginning of budding 5l/ha). In this variant, the yield capacity of dry raw materials was 27.7 cwt/ha, which is 4.2 cwt/ha more than in the control variant and 2.7 cwt/ha more than the variant with Stymovit Ferti. Such a low yield capacity in the control variant is also due to poor regrowth and tillering of plants.

The genetic potential of the productivity of medicinal plants is realized depending on the provision of plastic and energy resources, with nutrients playing an important role. More than 90% of plant biomass is synthesized during photosynthesis, that is why it is the main process for plant organisms that determines their productivity. The leading role in light absorption during photosynthesis belongs to chlorophylls, among which the main one is chlorophyll *a*. Carotenoids act as additional pigments during light absorption, in addition, they protect chlorophyll molecules

from irreversible photooxidation. Proper formation and functioning of photosynthetic structures occurs when plants are provided with nutrients, namely nitrogen and phosphorus.

Table 5

Yield capacity of dry raw material (leaves) of the medicinal plant *Mentha piperita* depending on the impact of complex fertilizers

Complex fertilizers	Norms and terms of applying fertilizers			Yield capacity of dry raw material (leaves)	
	Application of fertilizers for pre-sowing cultivation (kg/ha active substance)	Spraying plants in the germination phase (on 1 ha)	Spraying plants in the budding phase (on 1 ha)	cwt/ha	+; - compared to the control
1. Control - (without fertilizers)	-	-	-	18.2	-
2. Ecolyst	-	5 l	5 l	20.3	+2.1
3.Nitroamofoska + Ecolyst	$N_{30}P_{30}K_{30)}$ -	- 5 l	- 5 l	22.4	+4.2
4. Stymovit Ferti	-	5 l	5 l	18.7	+1.5
5.Nitroamofoska + Stymovit Ferti	$N_{30}P_{30}K_{30)}$ -	- 5 l	- 5 l	20.8	+2.6
LRD (the least real difference) 0.5				1.6 cwt/ha	

To study the effect of complex mineral and organic fertilizers on the biochemical composition of the photosynthetic apparatus of plants *D. canadense* and *M. piperita*, a comprehensive study of the pigment composition of the leaves of the studied plants under different cultivation conditions was carried out.

A comprehensive analysis of the pigment composition of leaves of *D. canadense* plants cultivated by applying complex mineral and organic fertilizers allows us to state that the same trend in the impact of these fertilizers on plants as for *M. piperita* plants. We believe that this is determined by the influence of the chemical composition of the applied fertilizers, in particular nitrogen and phosphorus, which entering the plant cells cause changes in the photosynthetic apparatus. The results of the study showed that the content of chlorophyll a in the leaves of the studied plants *D. canadense* varied in the range of 1.50-1.79 mg/g, chlorophyll *b* – in the range of 0.38-0.45 mg/g, and carotenoids - in the range of 0.52-0.61 mg / g of raw mass (Fig. 1). It was found that significantly the highest ($p < 0.05$) rates – 16.2%, 15.1% and 14.4%, respectively, higher than the plants grown in the control – were recorded in plants cultivated with applying of Nitroamofoska complex + Ecolyst. It was found out that in the control variant the ratio of chlorophyll *a / b* was 3.87 ± 0.24, and the sum of chlorophyll / carotenoids was 2.68 ± 0.18. Complex mineral and organic fertilizers did not affect these indices.

A study of the content of photosynthetic pigments in the leaves of *M. piperita* plants during their cultivation showed that the application of complex mineral and organic fertilizers had an effect on their content. The content of pigments in the control was: chlorophyll *a* – 1.18 ± 0.07 mg/g, chlorophyll *b* – 0.32 ± 0.02 mg/g, the amount of chlorophyll *a* and *b* – 1.49 ± 0.08 mg/g, carotenoids – 0.35 ± 0.03 mg/g of raw weight. Analysis of the effect of complex mineral and organic fertilizers during the cultivation of plants showed that their application increased chlorophyll a from 8.6 to 21.5%, chlorophyll *b* – from 6.6 to 22.4%, carotenoids – from 8.9 to 20.7% compared to control.

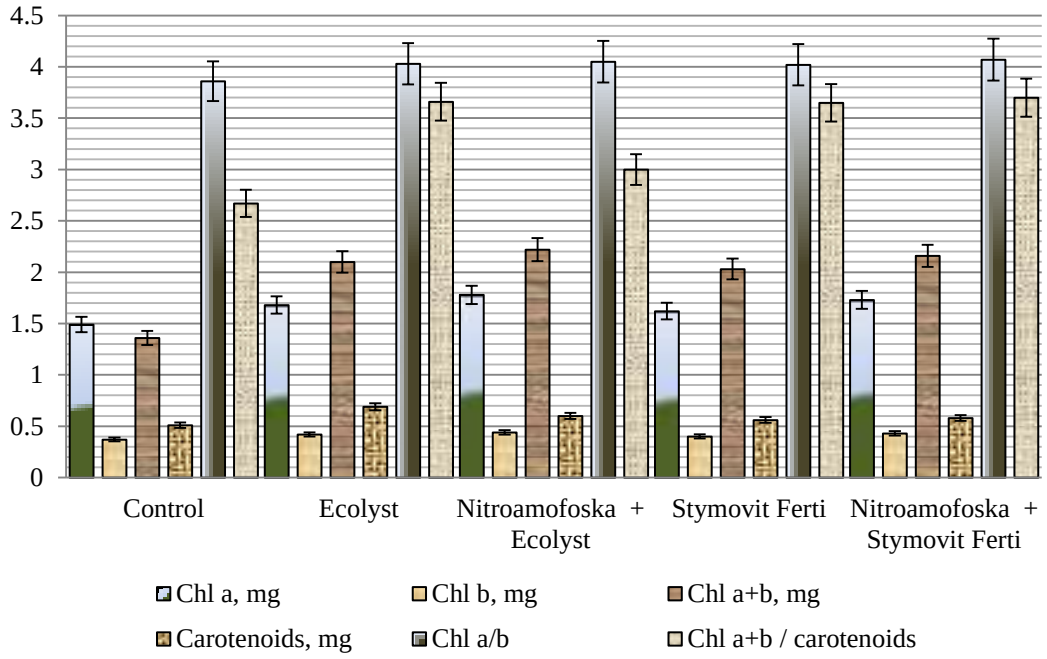

Fig. 1. The content and ratio of pigments in the leaves of Desmodium canadense plants under different cultivation conditions

Significantly the highest ($p < 0.05$) content of chlorophyll *a* and *b* and carotenoids compared to the control are characterized by plants grown by applying Nitroammophoska for pre-sowing cultivation and double application of complex fertilizer Ecolyst in phenological phases of germination and budding of plants (Fig. 2). It was found out that the content of chlorophyll a in the leaves of plants cultivated with applying of the complex Nitroamofoska + Ecolyst increased by 21.5%, and with applying of the complex Nitroamofoska + Stymovit Ferti – by 17.2%. The chlorophyll b content in these variants increased by 22.4% and 16.2%, respectively. The amount of carotenoids also increased with application of the preparations mentioned above by 20.7% and 17.7%, respectively, compared to the control. The increase of the content of photosynthetic pigments, in our opinion, indicates the high adaptive capacity of plants, which are mainly realized through the efficient operation of the photosynthetic apparatus.

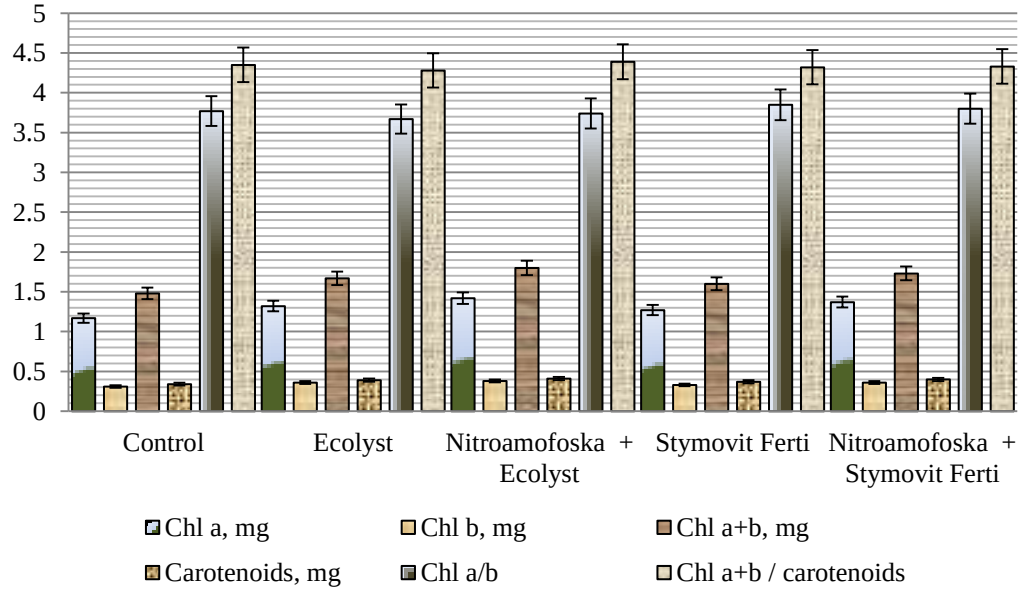

Fig. 2. Content and ratio of pigments in the leaves of Mentha piperita plants under different cultivation conditions

Taken inton account the greater importance of the ratio of pigments for the photosynthesis than their quantitative content, whereas this may indicate certain disturbances in the functioning of light collection complexes, as well as the reaction centers of photosystems, these indices were studied. It was found out that in the control variant the ratio of chlorophyll *a / b* was 3.78 ± 0.19, and the sum of chlorophyll / carotenoids was 4.36 ± 0.22. It was established that complex mineral and organic fertilizers did not affect the ratio of chlorophyll *a / b* and the amount of chlorophyll / carotenoids, hence, do not disrupt the functioning of plant photosystems.

Thus, the studied plants *M. piperita* and *D. canadense*, grown with complex mineral and organic fertilizers are characterized by significantly higher ($p < 0.05$) content of photosynthetic pigments compared to plants grown in the control, with the same ratios as in control plants chlorophyll *a / b* and the sum of chlorophyll / carotenoids. This indicates the high adaptive capacity of plants, which are mainly realized due to the efficient operation of the photosynthetic apparatus and the non-violation of the functioning of their photosystem. The highest rates were recorded in plants grown with applying of the complex Nitroamofoska + Ecolyst.

Conclusion

Studies have shown that the complex fertilizers Nitroamofoska, Ecolyst and Stymovit Ferti affected the morphological indices of the medicinal plants *D. canadense* and *M. piperita*. The best these onesi were found in the variants with applying Nitroamofoska ($N_{30}P_{30}K_{30}$) under pre-sowing cultivation and subsequent applyong Ekolyst and Stymovit Ferti fertilizers in two terms: the first time – when spraying plants in the germination phase (regrowth) and the second time – when spraying plants at the beginning), which significantly affected the height of plants, the number of shoots per plant, increasing the number of leaves per plant, stem thickness and length of the flowering part of the plant (inflorescence).

Nitroamofoska, Ecolyst and Stymovit Ferti complex fertilizers also had a significant effect on the yield capacity of medicinal plants (*D. canadense* and *M. piperita*). The highest yield one of raw materials (herbs, leaves) of medicinal plants *D. canadense* and *M. piperita* was set in the variant with the applying Nitroamofoska ($N_{30}P_{30}K_{30}$) under pre-sowing cultivation and application of Ekolyst fertilizer in two terms (the first time – when spraying plants in the regrowth phase 5 l/ha and the second time – when spraying plants at the beginning of budding 5 l/ha).

The studied plants *M. piperita* and *D. canadense*, grown with applying complex mineral and organic fertilizers are characterized by significantly higher ($p < 0.05$) content of photosynthetic pigments compared to the plants grown in the control, with the same as the control ones with chlorophyll a / b and the sum of chlorophyll / carotenoids. This indicates the high adaptive capacity of plants, which are mainly realized due to the efficient operation of the photosynthetic apparatus and the non-violation of the functioning of their photosystem. The highest rates were recorded in the plants grown with applying the complex Nitroamofoska + Ecolyst.

Therefore, medicinal plants *D. canadense* and *M. piperita* are best cultivated in the soil and climatic conditions of Precarpathian region of Ukraine with applying Nitroamofoska ($N_{30}P_{30}K_{30}$) for pre-sowing cultivation and subsequent application of fertilizers Ecolyst and Stymovit Ferti in two terms (the first time – when spraying plants in the germination phase and the second time – when spraying plants at the beginning of budding).

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Chapter 11. THE HAMBURG SCORE OF PROFESSOR YURI SHUNIN

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Abstract. *The Hamburg score is determined by the totality of the scientist's results, which influenced the development of the relevant scientific directions and turned out to be useful for solving related tasks by other scientists. The article gives a brief overview of the scientific results of Professor Yuri Shunin.*

Keywords*: Professor Yuri Shunin, the main scientific results.*

The scientific activity of Professor Yuri Shunin covered a wide range of areas and at various times affected the problems associated with the latest achievements of world science. There are three main directions in which he obtained significant results that affected progress in the relevant areas: theory of disordered solids, properties and applications of nanostructures, mathematical methods in pedagogy and linguistics.

In the doctoral dissertation of Professor Yuri Shunin the multiple scattering theories and the effective media cluster approach were developed with respect to disordered solids. The electronic structure of different disordered systems was studied. Later these approaches were used to solve many problems of solid-state theory and applied problems of modern electronics.

Thedevelopedclusterapproachfornumerical modelling of disordered solids, based on the multiple scattering theories, cluster conception and effective medium model allowed obtaining important results on the electronic structure of amorphous semiconductors. A number of works were carried out in this direction. The electronic density of states, a phonon density of states, a thermal conductivity and other properties necessary for practical applications were calculated.

The peculiarities of electrical properties of series of novel solid state devices were studied. The mechanism of resistivity was considered as a scattering problem, where the current carriers participate in the transport according to various mechanisms based on the presence of scattering centers (phonons, charge defects, structural defects, etc.). The developed computational procedure is based on the construction of cluster potentials and the evaluation of the S - and T- matrices for scattering and transfer, respectively. It allowed to realize the full-scale electronic structure calculations for condensed matter ("black box"), where influence means a set of electronic "trial" energy-dependent wave functions that gives sets of scattering amplitudes corresponding to possible scattering channels for any "trial" energy. This allowed "decrypting" the electronic spectra of "black box".

The second direction of an activity of professor Yuri Shunin is devoted to research of nanostructures, in particular nanotubes (CNTs) and graphene nanoribbons (GNR). His research was focused on the junctions of carbon nanotubes (CNTs) and graphene nanoribbons (GNR) with contacting metallic elements of a nanocircuit. Computer simulations on the conductance and resistance of these contacts have been performed using the multiple scattering theory and the effective media cluster approach. It was simulated both single-wall and multi-wall CNTs as well as

single- layered and multi-layered GNRs with different morphology. The model of CNT-Me and GNR-Me nanointerconnects (Fig. 1) has been developed. The electron transport formalism was used which suggests the existence of two regions supporting two different electron transport mechanisms: ballistic (elastic) and collisional (non-elastic). These electron transport processes are simulated using the corresponding boundary conditions in the form of the effective medium. The CNT and GNR chiralities (m, n) are simulated by the corresponding orientation of the chirality vectors within the scattering medium.

Figure 1 represents the contacts of metal substrates with CNTs and GNRs, respectively, as prototype nanodevices. The contact regions (CNT-Me and GNR-Me) are the objects of a microscopic approach responsible for the main contribution to the resistance. The resistances of nanotubes, nanoribbons and the metallic substrate per se are considered as macroscopic parameters.

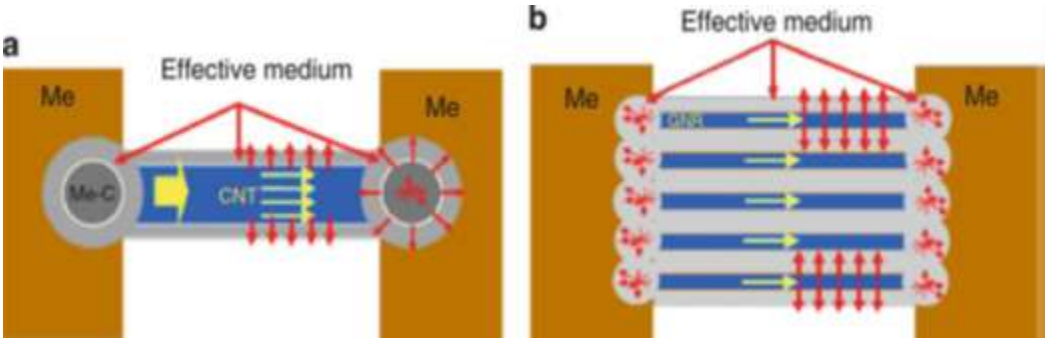

Fig. 1. Models of C-Me interconnects as a prototypes of novel nanodevices: (a) CNT- Me interconnect; (b) multilayered GNR-Me interconnect

The electronic structure for CNT-Me and GNR-Me interconnects was evaluated through the electronic density of states (DOS) for carbon-metal contact considered as a “disordered alloy”, where clusters containing both C and Me atoms behave as scattering centers. The computational procedure that was developed for these calculations is also based on the construction of cluster potentials and the evaluation of both scattering and transfer matrices. The general model of multiple scattering using the effective media approximation (EMA) combined with the coherent potential approach (CPA) for condensed matter was developed. When using the CPA as EMA approximation, the resistance of the interconnect was evaluated through Kubo-Greenwood formalism, or in the simplest cases, through Ziman formalism. So far, thecluster formalism has been successfully applied for Cu metal, as well as for semiconductors. Special attention was paid for the latter, since the concept of statistical weighing has been applied for the binary components in solid solutions. Structural models for CNT-Me and GNR-Me junctions were developed, based on their precise atomistic structures, which take into account the CNT chirality effect and its influence on the interconnect resistance.

The most problematic areas for simulation of CNT-Me and GNR-Me were the necessity to account that there is the atomic structural disorder and the conductivity mechanism changing. The influence of chirality on resistance in the vicinity of interconnect depends on the number of statistically realized bonds between the CNT (GNR) and the metalcontact (e.g., Ni, Cu, Au, Ag, Pd, Pt). For the case of side type contact for GNR-Me interconnects the model of effective bonds was developed and it was shown that the number of effective bonds per contact square is essential.

It was accounted in the calculations of interconnects that here a probabilistic process occurs when only more-or-less equilibrium bonds (‘effective bonds’) are formed at inter-atomic distances corresponding to the minimum total energies. The evaluation of a number of “effective bonds” is principal for determination of the number of ‘conducting channels’, since the conductance is proportional to the number of appeared “effective bonds” within the CNT-Me interconnect. The features of contacts were studied in detail.

It was established that the radial conductance per CNT length depends on the morphology (chirality) of the nearest nanotubes, when the number of shortest effective barriers is varied in a probabilistic way. This also means that current-voltage parameters of contacts can be less stable. It has been found that inter-shell interactions, such as inter-shell tunneling of electrons and Coulomb interactions cause a reduction of the total conductance in considered contacts.

A large cycle of works was performed by Professor Yuri Shunin in the field of research and creation of fundamentally new biosensors based on previously studied nanostructures. These results are connected with applications of CNTs and GNRs based interfaces with other materials. When creating biosensors, the well known electron devices, field-effect transistors (FET-transistors) (Fig. 2) were used which are very sensitive to various external influences of different nature such as mechanical, chemical, electrical, magnetic etc. A FET is of nano in size, whose on/off threshold voltage depends on the tube dimensions, shape and temperature, amongst others. A local deformation of CNT (GNR) creates a change in the on/off threshold voltage of the transistor. The electrical properties of carbon based interconnects are changed under the influence of different external factors. The advantage of CNTs and GNRs over other structures occurs due to their small size, great strength, high electrical and thermal conductivity, and high specific area. Unique physical properties of CNTs and GNRs and their various interconnects allow considering them as sensing nanomaterials in various kinds of sensors – pressure, flow, thermal, gas, optical, mass, position, stress, strain, chemical, and biological sensors. Taking into account specific physical properties of CNTs and GNRs metal interconnects which are explained by the presence of “dangling” chemical bonds, it was pointed out the expressed sensitivity of electric properties of interconnect space to chemical, electric and magnetic factors’ influence. Thus it was shown that interconnects in the mentioned devices are important elements for a perspective group of nanosensors.

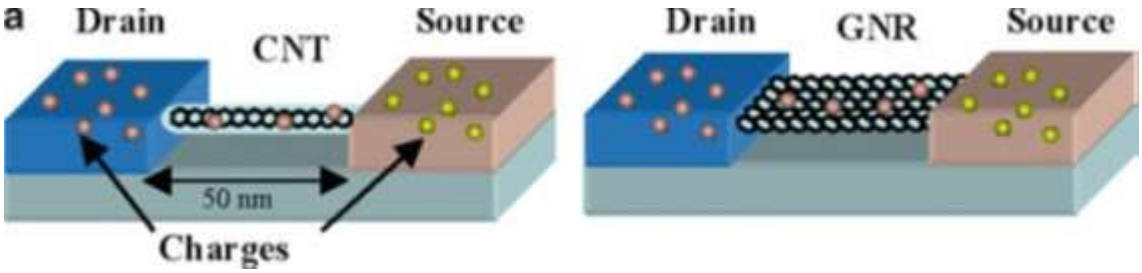

Fig. 2. The unperturbed field-effect transistors based on a CNT and GNR are given CNT- or GNR- based FET. They are mainly composed of a corresponding semiconducting carbon material suspended over two electrodes

Further, the biological nanosensors based on the use of noble metal nanoparticles were investigated. Mechanism of sensing is connected with an excitation of surface plasmons. The resonant frequency of surface plasmons that are caused by the light irradiation changes when different harmful components are adsorbed on the particles. This technique based on so called localized surface plasmonresonance (LPSR) is widely used. One of the main constraints of this sensing mechanism is the requirement of an external source of light and a device which is able to measure and compare different resonant frequencies.

In the works of Professor Yuri Shunin various classes of CNT- and GNR-based nanosensors are considered. A wide class of biological nanosensors is investigated for theapplication in the biomedical industry, e.g., in cases of diabetes, where regular tests by patients themselves are required. This includes many other diagnostic and therapeutic devices. For example, these are devices such as biosensors for application in eye surgery, hospital beds equipment, patient monitors, inhalers, and kidney dialysis machines, in both invasive and noninvasive blood pressure monitoring. Nanosensors were considered that have a substantial utility in the automotive industry. Their importance is expected to increase while designing the vehicles of the future. In particular,

they are used to process information about vehicle parameters such as pressure, vehicle altitudes, flow, temperature, heat, humidity, speed and acceleration, exhaust gas, and engine knock and torque.

In recent years, Professor Yuri Shunin has developed a new direction related to the application of mathematical methods in pedagogy and linguistics. Some new definitions to pedagogical phenomena in the process of language acquisition were proposed on the basis of the general systems theory. A group of learners was considered as a learning system which is reversely charged with a situational managerial system (i.e. mentoring/teaching staff), thus, forming a constituent structural unit of a bigger pedagogical system but keeping at the same time all its main characteristics. Since the learning system experiences a purposeful external pedagogical influence, it is considered a managed system. A model of Intelligent System Management has been worked out. The principles developed are adequate also for other study activities and study courses. The process of imparting educational information by a mentor is distinguished by its qualitative and quantitative indices. It was considered a process of intellectualization of a study group in connection with the notion of “Homeokinetic Plato”’, which actually reflects different intellectual levels of tested study group, e.g., in a language acquisition. The proposed System of Intelligence Levels and the Teaching Efficiency Indicator ensure the possibility to estimate the initial level of learner intelligence and the final result and compare these results with a predetermined purposeful goal to see the efficiency of a study course and the progress of student achievement. These techniques can be recommended for use to a variety of educational domains. An empirical study was used to analyze the optimum amount of the language material to be included into the final test on Business English. The empirical results gave grounds to compile effectively the examination paper material amount and to define the time for its fulfilment. Optimization Model of teaching information amount and time distribution has been worked out. The system approach to language teaching and acquisition allows removing the blinders so that it’s possible to see the educational world in the light that illuminates the whole – the system – and only then there will be lasting changes for the better.

Conclusion

The results obtained by Professor Yuri Shunin left a significant mark on important fields of science. These results are in many cases obtained with the participation of colleagues and students. Cooperation and the exchange of ideas were the leading principle in the creative work of Professor Yuri Shunin. The ideas embodied in these works will undoubtedly be further developed.

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