XXXI. KRKINE NAGRADE
XXXIST KRKA PRIZES

XI. mednarodni simpozij / International Symposium

Novo mesto, Slovenija
25. in 26. oktober 2001

KRKA

Častni odbor XXXI. KRKINIH NAGRAD

Miloš Kovačič, predsednik uprave in generalni direktor Krke, predsednik
France Bernik, predsednik Slovenske akademije znanosti in umetnosti
Jože Mencinger, rektor Univerze v Ljubljani
Ludvik Toplak, rektor Univerze v Mariboru

Svet SKLADA KRKINIH NAGRAD

Aleš Rotar, predsednik
Miha Japelj
Jože Drinovec
Natalija Vitezić
Stanislav Zalar
Alenka Pučko
Ivan Radež
Janja Požar
Dušan Dular
Aleš Hvala
Jože Gnidovec
Irena Čarman
Damjan Možina

Organizacijski odbor za podelitev nagrad

Alenka Pučko, predsednica
Janja Požar
Mateja Vukšinič
Dušan Dular
Suzana Hočevar
Danilo Škedelj

Recenzenti

Zvezdana Bajc, Milan Banovec, Pija Berus, Milan Bezeg, Marko Boh, Marjeta Brumen, Branka Cencelj, Nina Cimerman, Lea Cvitkovič Maričić, Irena Čarman, Silvo Derganc, Jože Drinovec, Dušan Dular, Vesna Flego, Aleš Gasparič, Dora Glažar, Bogomir Golobič, Jože Gnidovec, Mateja Gorjup, Janez Hočevar, Aleš Hvala, Marijan Ivanuša, Miha Japelj, Jernej Jedlovčnik, Brane Kastelec, Bojan Kebe, Saša Kolarič, Majda Kopač, Janko Kos, Berta Kotar-Jordan, Janez Kržan, Andreja Kuhar, Borut Lekše, Matej Luštek, Alenka Malenšek, Mario Merslavič, Peter Miklavčič, Anita Mlakar, Damjan Možina, Irena Orel, Branko Pavlič, Mitja Pelko, Robert Pišek, Aleš Plaper, Andreja Plaper, Miroslav Pokorny, Vladimir Pokorny, Janez Poljanec, Mojca Prah Klemenčič, Jože Primc, Alenka Pučko, Ivan Radež, Anton Recelj, Slobodan Rekič, Aleš Rotar, Miloš Ružič, Janez Smodiš, Elizabeta Suhadolc, Božena Šuštar, Jaroslav Tihi, Boris Veselič, Natalija Vitezić, Vesna Vočanec, Polona Vodopivec, Franc Vrečer, Mateja Vrečer, Stanislav Zalar, Janez Zupančič, Slavko Zupančič, Pavle Zupet, Rok Zupet.

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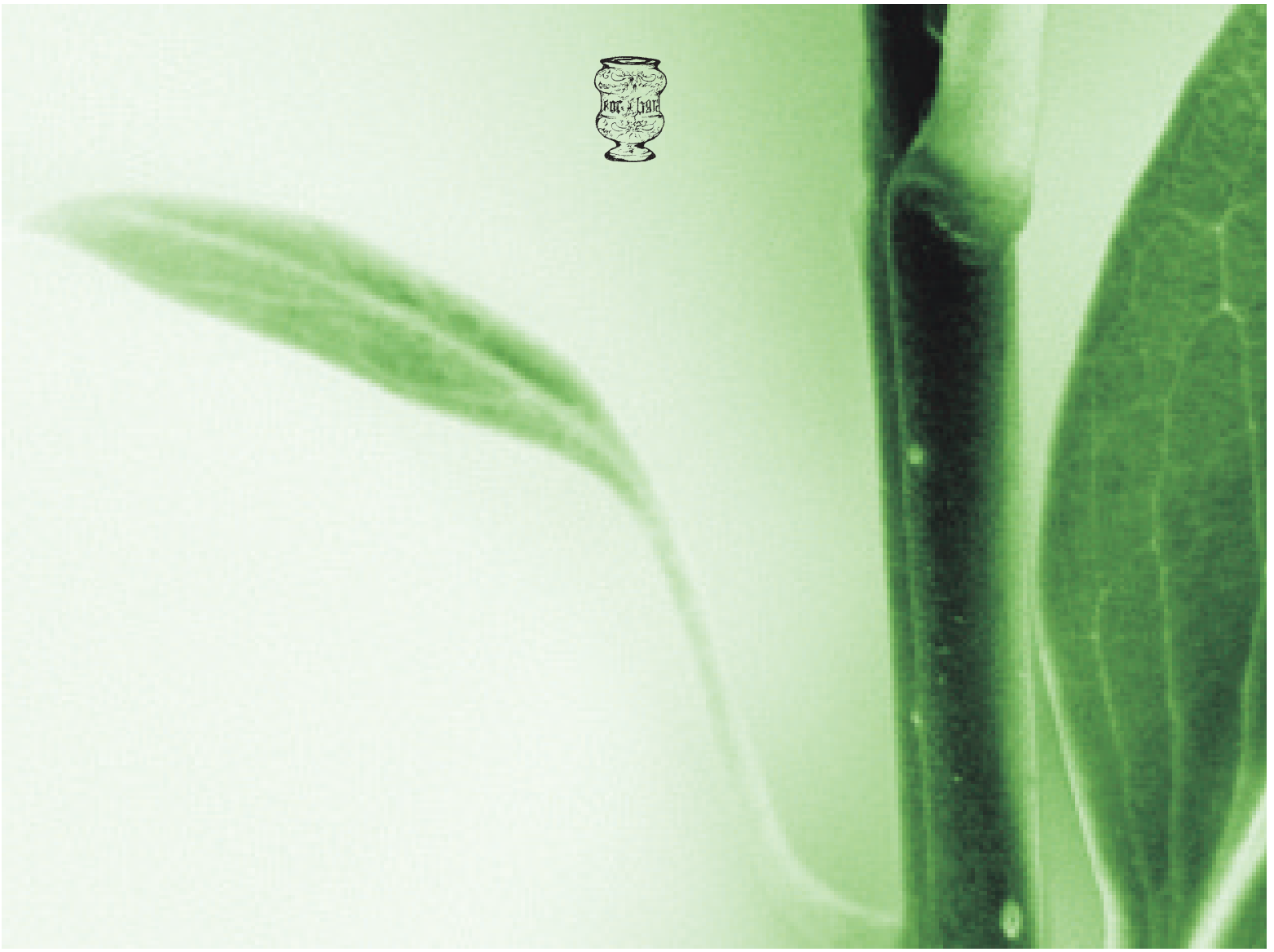


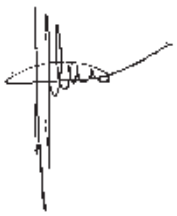
XXXI. KRKINE NAGRADE

UVODNE BESEDE

Krkine nagrade, enaintridesete po vrsti, podeljujemo v času, ko smo na začetku tretjega tisočletja soočeni z vrsto velikih izzivov. Na prehodu iz ene tehnološke revolucije v naslednjo, še neoprijemljivo in zavito, v negotovost, je bolj kot kdajkoli jasno, da bo znanje glavno gibalo razvoja. Ko v Krki gledamo v prihodnost, vidimo zapleteno in težavno pot. Prav take pa si tudi želimo, saj tržnega uspeha v sodobni ekonomiji ni moč dosegati z reševanjem preprostih nalog. Zavedamo se, da je zato pomembno združevati izkušnje in mladost, pretekle uspehe in najnovejše teorije, preudarnost in željo po tveganju. Zato vidimo Krkine nagrade kot forum študentov, mladih raziskovalcev in njihovih mentorjev iz akademskih krogov ter Krkinih strokovnjakov. Želimo si, da bi se spoznavali, saj je od razumevanja in prepoznavanja sinergij odvisna naša skupna uspešnost. Predvsem pa je za to potrebna želja dosegati cilje. Tisti, ki ste si jih najbolj želeli doseči, ste danes nagrajenci, vam gredo posebne čestitke. Mentorjem in profesorjem smo dolžni zahvalo za njihovo znanje in zlasti za vztrajno vzpodbujanje k raziskovanju novega. Za nas vse pa velja: iščimo naprej, raziskujmo, da bi vedeli več in da bi nam zato bilo bolje.

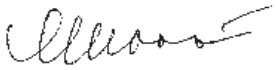
Predsednik Sveta
Sklada Krkinih nagrad

Dr. Aleš Rotar



Predsednik uprave in
generalni direktor Krke

Miloš Kovačič, mag. farm.



OPENING WORDS

We are presenting the XXXIst Krka's prizes at the beginning of 3rd Millenium, when we are all confronted with a number of great challenges. This is the time of transition from one technological revolution into another, which is still vague and uncertain, and it is more evident than ever before that knowledge will be the main drive of development. When we, at Krka, are focusing on the future, we see a complicated and difficult way ahead. However, this is exactly what we wish as market success in modern economy cannot be achieved by solving simple tasks. We are well aware that it is very important to combine experience and youth, past achievements and the latest theories, prudence and desire to take risks. For this reason, we see Krka's prizes as a forum of students, young researchers and their mentors from the academic circles and Krka's experts. We wish you could get acquainted with each other as our common successfulness depends on understanding and recognizing synergies. But the most important thing of all is the desire to reach the set goals. Those of you who wanted to reach them most have succeeded as award winners and you deserve special congratulations. We owe the mentors and professors many thanks for their knowledge and especially persistent encouragement for new researches. However, the following motto holds true for all of us: go on with searching and researches in order to know more and to have a better life because of this.

President of
Krka Prizes Foundation

Aleš Rotar

Chief Executive and
the President of the Management Board

Miloš Kovačič

KRKINI NAGRAJENCI 2001

	NAGRAJENCI	STATUS	MENTORJI
1702	ZRINKA ABRAMOVIĆ	študentka farmacije	Julijana KRISTL Marjeta ŠENTJURC
1703	MARUŠA AVSEC	študentka biologije	Mihael J. TOMAN
1704	ANDREJ BABIČ	študent farmacije	Slavko PEČAR
1705	ŠPELA BAEBLER	podiplomska študentka biologije	Jana ŽEL
1706	NINA BANČOV	študentka družbenih ved	Marko LAH Damjan MOŽINA
1707	DANICA BARBO SIMONIČ	študentka informatike	Vladislav RAJKOVIČ
1708	ANELA BEŠO	dijakinja	Tomaž ŠAVELJ Ingrid POVŠE Tatjana DURMIČ
1709	MATEJ BIZJAK	študent biotehnologije	Romana MARINŠEK LOGAR Aleš GASPARIČ
1710	NENAD BJELAN	študent farmacije	Borut ŠTRUKELJ Damjana ROZMAN
1711	IRENA BOBNAR	študentka farmacije	Aleš MRHAR Jože DRINOVEC
1712	ZLATKO BOJANOVIČ	študent kemijske tehnologije	Danilo DOBČNIK
1713	PETRA BUKOVEC	dijakinja	Marinka KOVAČ
1714	DMITRY V. CHUVIKOVSKY	študent biokemije, Rusija	
1715	JOŽE DERGANC	študent elektrotehnike	Franjo PERNUŠ
1716	ANJA DULAR	dijakinja	Tomaž ŠAVELJ Tatjana DURMIČ Ingrid POVŠE
1717	PETRA DULAR	študentka likovne umetnosti	Miro KLINE Peter SKALAR
1718	PETR FRÁNA	podiplomski študent medicine, Češka	
1719	VESNA GALVANI	doktorandka	Vladka ČURIN ŠERBEC
1720	MOJCA GOLOB	podiplomska študentka biokemije	Roman JERALA
1721	MARICA GRDIN	študentka farmacije	Robert UCMAN
1722	IGOR HES	študent kemijske tehnologije	Jana ZAGORC KONČAN Slavko ZUPANČIČ
1723	MIHA HOMAR	študent farmacije	Mirjana GAŠPERLIN
1724	ANAMARIJA HRAST	študentka družbenih ved	Marko LAH Damjan MOŽINA
1725	JANEZ ILAŠ	študent farmacije	Slavko PEČAR
1726	NATALIJA JANTOLEK	študentka ekonomije	Borut BRATINA Drago FILIPIČ
1727	SANDRA JAVERNIK	študentka farmacije	Samo KREFT
1728	IVANKA JERIĆ	doktorandka, Hrvaška	Štefica HORVAT
1729	MARJAN JEŠELNIK	podiplomski študent kemije	Marijan KOČEVAR
1730	ANDREJ KASTELIC	študent zootehnike	Andrej ŠALEHAR Silvester ŽGUR

1731	MATEJA KOCJAN	študentka farmacije	Aleš ROTAR Andrejka KRAMAR Franc VREČER
1732	ANDREJKA KRAMAR	podiplomska študentka farmacije	Franc VREČER
1733	SREČKO MITJA KRIŽNIČ	podiplomski študent kemijske tehnologije	Milenko ROŠ
1734	VESNA KROŠELJ	študentka kemije	Branko STANOVNIK Jurij SVETE
1735	MARIUSZ KRUK	doktorand, Poljska	
1736	NATAŠA KUZMA	študentka ekonomije	Franc LORBEK Damjan MOŽINA
1737	MITJA LAINŠČAK	podiplomski študent medicine	Irena KEBER
1738	ROMAN LENARŠIČ	doktorand	Slovenko POLANC
1739	ANDREJA MAJERLE	doktorandka	Roman JERALA
1740	MAJA MARKOČIČ	študentka farmacije	Marija SOLLNER DOLENC
1741	IRENA PETRIŠIČ	študentka farmacije	Božena ŠUŠTAR Robert PIŠEK
1742	ALEŠ PREMZL	podiplomski študent medicine	Janko KOS
1743	IZTOK PUSTATIČNIK	podiplomski študent ekonomije	Marko HOČEVAR
1744	VIDA REZAR	študentka zootehnike	Janez SALOBIR Romana MARINŠEK LOGAR
1745	ZORAN RODIĆ	dijak	Irena HOČEVAR Igor PLAPER
1746	ZVONE SIMONČIČ	študent farmacije	Danijel KIKELJ Matej BREZNIK Janez PLAVEC
1747	TOMAŽ SMRKOLJ	študent medicine	Zoran GRUBIČ Marjan RUPNIK
1748	VATROSLAV SPUDIĆ	študent biotehnologije	Romana MARINŠEK LOGAR Aleš GASPARIČ
1749	BOŠTJAN ŠIMUNIČ	študent elektrotehnike	Vojko VALENČIČ
1750	IRENA ŠUTAR	dijakinja	Marinka KOVAČ
1751	ELIZABETA TRATAR PIRC	doktorandka	Peter BUKOVEC
1752	TOMAŽ TRČEK	podiplomski študent kemije	Bojan VERČEK
1753	UROŠ VIDIC	dijak	Irena HOČEVAR Igor PLAPER
1754	IGOR VIZEC	dijak	Marinka KOVAČ
1755	JULIJA VOLMAJER	študentka kemije	Alenka MAJCEN LE MARECHAL Shmuel BITTNER
1756	URŠKA ZAVEC	študentka farmacije	Robert PIŠEK Franc VREČER
1757	DAVID ZUPANČIČ	študent ekonomije	Vesna ŽABKAR Natalija VITEZIĆ
1758	SILVO ZUPANČIČ	doktorand	Branko STANOVNIK
1759	LEA ŽBONTAR ZVER	podiplomska študentka kemijske tehnologije	Peter GLAVIČ
1760	NATAŠA ŽMUC	študentka farmacije	Darja BARLIČ MAGANJA

XI. mednarodni simpozij
International Symposium

Zbornik povzetkov
Book of Abstracts

Novo mesto, Slovenija
25. oktober 2001

KRKA

Znanstveni odbor XI. mednarodnega simpozija

Miha Japelj, predsednik
Aleš Gasparič
Franc Vrečer
Janko Kos
Saša Kolarič

OB XXXI. KRKINIH NAGRADAH ŽE XI. MEDNARODNI SIMPOZIJ

Krkine nagrade so posvečene znanju, ustvarjalnosti in spoznavanju pomembnih vrhunskih raziskav in dosežkov. Že kar lepo obdobje so slavnosti ob podelitvah Krkinih nagrad posebna priložnost, da se mladi študentje in dijaki pri nas v Krki srečajo s številnimi profesorji in učitelji, raziskovalci in strokovnjaki z univerz, številnih domačih in tujih inštitutov in iz farmacevtske industrije. Na teh srečanjih nastajajo nova poznanstva in prijateljstva, snujejo se novi načrti in nova sodelovanja. Prav zaradi vsega tega smo že pred enajstimi leti začeli Krkine nagrade povezovati z mednarodnim simpozijem. Nanj vabimo vrhunske znanstvenike, strokovnjake in raziskovalce s celega sveta. Obravnavamo najsodobnejše in raznovrstne znanstvene in razvojne teme, ki so ključnega pomena za našo farmacevtsko-kemijsko industrijo. V teh enajstih letih smo v Krko kot plenarne predavatelje povabili že 45 uveljavljenih vrhunskih univerzitetnih profesorjev, akademikov in strokovnjakov iz 18 držav Evrope in ZDA in prek 20 iz Slovenije.
Na mednarodnih simpozijih predstavljamo tudi raziskovalne dosežke Krkinih nagrajencev. S predavanji sodelujejo doktorji znanosti, drugi nagrajenci pa predstavljajo svoje dosežke v obliki posterjev. Letos smo medse kot plenarne predavatelje povabili priznane akademike, profesorje in strokovnjake iz šestih držav, iz Rusije, Švice, Poljske, Hrvaške, Češke in Slovenije.
Na XI. mednarodnem simpoziju, ki ga bo slavnostno odprl podpredsednik Slovenske akademije znanosti in umetnosti **akademik prof. dr. Alojz Kralj**, bodo sodelovali vabljeni plenarni predavatelji:

akademik prof. dr. Anatoly I. Mirošnikov iz Rusije

Akademik prof. dr. Anatoly I. Mirošnikov je namestnik direktorja inštituta za bioorgansko kemijo (Inštitut Šemjakina in Ovčinikova) ruske akademije znanosti. Profesor Mirošnikov, doktor kemijskih znanosti in član ruske akademije znanosti, spada med vrhunske ruske biokemike. V sodobnem inštitutu, ki ga je tudi sam moderno zasnoval, vodi in usklajuje vrsto ključnih raziskav s področja biokemije. Razvil je tehnologijo za polindustrijsko proizvodnjo rekombinantnega človeškega inzulina in razvija biotehnološko proizvodnjo oksitocina. Je avtor številnih publikacij (prek 200) in član pomembnih ruskih in mednarodnih združenj, predvsem s področja bioorganske kemije.

prof. dr. Robert Gurny iz Švice

Prof. dr. Robert Gurny je predstojnik Oddelka za farmacevtsko tehnologijo in biofarmacijo na Fakulteti za farmacijo Univerze v Ženevi. Vrsto let je deloval in se izpopolnjeval na univerzah v ZDA (Purdue, Standford) in Franciji. Spada med vodilne svetovne strokovnjake na področju sodobnih tehnologij za oblikovanje zdravil, posebno koloidnih nosilcev učinkovin. Zlasti pomembne in v svetu odmevne so njegove originalne in pionirske raziskave pri načrtovanju, sintezi in uporabi biološko razgradljivih polimerov. Profesor Gurny je avtor številnih znanstvenih in strokovnih člankov (več kot 200), patentov (20) ter pomembnih znanstvenih in strokovnih knjig. Je urednik European Journal of Pharmaceutics and Biopharmaceutics. Vrsto let je bil tudi generalni tajnik strokovnega društva Controlled Release Society v ZDA. Za svoje vrhunske dosežke je prejel več prestižnih mednarodnih nagrad. Od leta 1995 uspešno sodeluje s Fakulteto za farmacijo Univerze v Ljubljani.

prof. dr. Witold Ružyłło iz Poljske

Prof. dr. Witold Ružyłło je vrhunski poljski in svetovni kardiolog. Je vodja oddelka za kardiologijo na poljskem državnem inštitutu za kardiologijo. Kot vrhunski strokovnjak uživa velik ugled doma in po vsem svetu. Vrsto let je aktivno deloval v Kanadi in ZDA. Je član poljskih, evropskih in svetovnih medicinskih združenj za kardiologijo in avtor številnih znanstvenih člankov in knjig. Posebej je treba izpostaviti vlogo koordinatorja v velikih mednarodnih kliničnih programih, kot so CAPE II, PURSUIT, GUSTO IV in ALIVE.

prof. dr. Ante Simonić iz Hrvaške

Prof. dr. Ante Simonić je vodilni hrvaški farmakolog. Znanstveno in strokovno se je usposabljal v Belgiji, Angliji in v ZDA. Je redni profesor farmakologije na Reki in gostujoči profesor v Belgiji. Ima pomemben položaj v hrvaškem državnem zboru. Osnovne raziskave profesorja Simonića so usmerjene v farmakologijo, toksikologijo, posebej v nevrofarmacijo in sodobno metodologijo razvojno-raziskovalnega dela. Je član številnih mednarodnih in hrvaških društev in medicinskih združenj ter mislec in vizionar, ki v svojih zadnjih knjigah razglablja o etiki, kulturi, znanosti in o smislu nastanka in obstoja človeštva, ki je krona geneze.

doc. dr. Renata Cifková iz Češke

Doc. dr. Renata Cifková vodi oddelek za preventivno kardiologijo na Inštitutu za klinično in eksperimentalno medicino v Pragi. Vidno in izrazito se v svetu in doma uveljavlja na področju preventivne kardiologije in klinične farmakologije. Usposabljala se je na Japonskem, v Kanadi, Nemčiji in v ZDA. Osnovne smeri njenih raziskav so epidemiologija ateroskleroze in arterijske hipertenzije, hormonsko zdravljenje in druge. Je predsednica češkega društva za hipertenzijo ter članica izbranih mednarodnih komitejev in društev za kardiologijo in hipertenzijo.

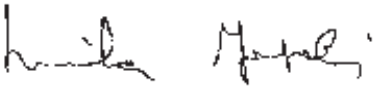
akademik prof. dr. Branko Stanovnik iz Slovenije.

Akademik prof. dr. Branko Stanovnik spada med vrhunske, svetovno priznane organske kemike. S svojimi publikacijami s področja heterociklične kemije (več kot 530), izbranimi patenti in številnimi knjigami se je vidno uveljavil v svetu. Izpopolnjeval se je v Kanadi, ZDA in v Avstraliji, kot vabljeni plenarni predavatelj je predaval v več kot dvajsetih državah. Je član številnih mednarodnih odborov, komitejev in združenj kemikov po vsem svetu. Je tudi pomemben član številnih znanstvenih in univerzitetnih ustanov v Evropi in ZDA. Za svoje dosežke je prejel številna visoka priznanja doma in v tujini. Profesor Stanovnik je bil mentor številnim Krkinim nagrajencem.

Svoja doktorska dela bodo predstavili tudi Krkini nagrajenci: Mariusz Kruk, Vesna Galvani, Andreja Majerle, Ivanka Jerić, Elizabeta Tratar Pirc, Roman Lenaršič, Silvo Zupančič.
V posebni sekciji pa bodo ostali Krkini nagrajenci v obliki posterjev predstavili raziskovalne dosežke iz svojega dodiplomskega in magistrskega študija. O raziskavah bodo govorili tudi najmlajši Krkini nagrajenci, dijaki iz dolenjskih srednjih šol.
Vsem vabljenim plenarnim predavateljem in vsem Krkinim nagrajencem se prisrčno zahvaljujemo, ker so s svojo ustvarjalnostjo prinesli v Krko novo znanje, novo kakovost in novo ustvarjalno moč, kar je v teh težkih časih tako zelo pomembno.

Predsednik znanstvenega odbora
XI. mednarodnega simpozija

prof. dr. Miha Japelj



AT THE XXXIST KRKA PRIZES ALREADY XITH INTERNATIONAL SYMPOSIUM

It is known that our Krka's Prizes are dedicated to knowledge, inventiveness and presentation of important outstanding research work and its results. For quite some years the Krka Prizes presentation ceremony has become an opportunity for young university students and medium school pupils to meet with numerous professors, teachers, researchers and professional experts coming from universities and several Slovenian and foreign institutes, and from the pharmaceutical industry. New acquaintances and friendships are made here, and new projects and plans for cooperation in the future are being created. This is why we decided, eleven years ago, to interlink Krka Prizes with the organisation of an international symposium. Every year we invite to these symposia renowned scientists, professional experts and researchers form all around the world. Discussions and lectures deal with a wide range of state-of-the-art research and development topics which are of key importance for our pharmaceutical and chemical science. Over the past eleven years we invited as plenary lecturers 45 renowned university professors, academicians and professional experts from 18 European countries and the US and also more than 20 distinguished experts from Slovenia.

At these international symposia we present research studies of the Krka Prize winners. Some of the prize winners present their doctoral theses in lectures, while others present their research results in the Poster section. This year, the plenary lecturers at the symposium are renowned academicians, professors and professional experts from Russia, Switzerland, Poland, Croatia, the Czech Republic and Slovenia.

At the XIth International Symposium, which will be officially opened by the Vice-President of the Slovenian Academy of Sciences and Arts,

Academician Prof. Dr. Alojz Kralj,

we will have the honour to greet our lecturers:

Academician Prof. Dr. Anatoly I. Miroshnikov from Russia

Academician Prof. Dr. Anatoly I. Miroshnikov is the Deputy Director of the Institute of Bio-organic Chemistry at the Russian Academy of Sciences (The Shemyakin and Ovchinikov Institute). Professor Miroshnikov, a member of the Russian Academy of Sciences is one of the Russian most important biochemists. He is one of the formers of this modern institute, where he conducts and supervises a number of key studies from the field of biochemistry. Professor Miroshnikov developed the technology for the semi-industrial production of the recombinant human insulin and is working on the development of biotechnological production of oxytocin. He published a great number of - over 200 - scientific publications and is a member of many Russian and international associations, primarily from the field of bio-organic chemistry.

Prof. Dr. Robert Gurny from Switzerland

Prof. Dr. Robert Gurny is the Head of the Department of Pharmaceutical Technology and Bio-Pharmacy at the School of Pharmacy of the University of Geneva. For many years he worked at universities in the US (Purdue, Standford) and France. He is one of the leading experts in the world in the field of contemporary technologies for drug formulations, particularly for colloidal carriers of active substances. His original and pioneer research work in the use of biodegradable polymers is of high importance and it evoked great interest in pharmaceutical industry. Professor Gurny's work also includes a number of patents (20), important scientific and expert books and over 200 scientific and expert articles. He is the editor of the European Journal of Pharmacy and Biopharmacy, and for several years, the Scientific Secretary of the Controlled Release Society. His important work has many times been awarded with significant international prizes.

Prof. Dr. Witold Ružyłło from Poland

Prof. Dr. Witold Ružyłło is the leading Polish, and an internationally acclaimed authority in cardiology. He is the Head of the Cardiology Department at the Polish State Institute of Cardiology. Being a foremost expert in his profession, he has gained great respect at home and abroad. For many years he also worked in hospitals and institutes in Canada and the US. Professor Ružyłło is a member of several Polish, European and international medical and cardiological associations and author of a great number of scientific articles and books. Particularly important is his role as the coordinator in some large international clinical programmes such as CAPE II, PURSUIT, GUSTO IV and ALIVE.

Prof. Dr. Ante Simonić from Croatia

Prof. Dr. Ante Simonić is the leading Croatian pharmacologist. He gained expert education during his work in Belgium, Great Britain and the US. He is a regular professor of pharmacology at the University of Rijeka, and a visiting professor at a university in Belgium. He also has an important position in the National Assembly of the Republic of Croatia. In his studies Professor Simonić focuses into pharmacology, toxicology and particularly into neuropharmacy and the modern methodology of research and development. He is a member of many international and Croatian societies and medical associations. Apart from his scientific engagement he is also a great thinker and visionary, developing his thoughts in his books on the ethics, culture, science and the sense and existence of the mankind as the crown of Genesis.

Assistant Professor Dr. Renata Cifková from the Czech Republic

Assistant Professor Dr. Renata Cifková is the Head of the Department of Preventive Cardiology at the Institute of Clinical and Experimental Medicine in Prague. Her important work in the science of preventive cardiology and clinical pharmacology made her well known in her home country and world-wide. Dr. Cifková studied in Japan, Canada, Germany and the US. Her main research topics include epidemiology of atherosclerosis and arterial hypertension, hormonal treatment and other. Dr. Cifková is the president of the Czech Society of Hypertension and a member of distinguished international committees and associations of cardiology and hypertension.

Academician Prof. Dr. Branko Stanovnik from Slovenia

Academician Prof. Dr. Branko Stanovnik is one of the foremost, internationally renowned experts in organic chemistry. He has won renown in the chemical science due to his over 530 publications on heterocyclic chemistry, selected patents and numerous books. He studied in Canada, the US and in Australia, and later as a plenary lecturer, he was invited to more than twenty countries. Professor Stanovnik is a member of many international committees and associations of chemists world-wide. He is also an important member of numerous European and American scientific and university institutions. His scientific work has been crowned with several prestigious prizes in Slovenia and abroad. Professor Stanovnik has also been a mentor to many Krka Prize winners.

The doctoral theses will be presented by this year's Krka Prize winners: Mariusz Kruk, Vesna Galvani, Andreja Majerle, Ivanka Jerić, Elizabeta Tratar Pirc, Roman Lenaršič, Silvo Zupančič.

Other prize winners will present their research work from their pre-graduate and master degree studies in the Poster section. In addition, the high school pupils from the Dolenjska region, who are the youngest Krka Prize winners, will present their research assignments.

We would like to thank sincerely to all the invited lecturers and all Krka Prize winners for contributing their innovative knowledge to Krka, bringing along with it new quality and creative power, which nowadays, in this difficult time, is of paramount importance.

Chairman of the Scientific Board
of the XIth International Symposium

Prof. Dr. Miha Japelj

PROGRAMME OF THE XITH INTERNATIONAL SYMPOSIUM

9.00-9.10 **Chairman's Welcome Address**

9.10-9.20 **Opening of the Symposium - Alojz Kralj**

1 Plenary Lectures - Part I.

Chairman: **Miha Japelj**

9.20-9.55 *Prospective for Development of Biotechnology-Based Pharmaceuticals*
Anatoly I. Miroshnikov **23**
Shemyakin & Ovchinnikov Institute of Bioorganic Chemistry,
Russian Academy of Sciences, Moscow, Russia

9.55-10.30 *Surface Eroding Biopolymers for Drug Delivery: Therapeutic Applications*
Robert Gurny **24**
School of Pharmacy, University of Geneva, Switzerland

10.30-11.05 *Techniques of Interventional Cardiology in the XXIst Century*
Witold Rużyłło **25**
National Institute of Cardiology, Department of General Cardiology,
Warsaw, Poland

11.05-11.40 *α,β-Unsaturated Acid Derivatives and Their Chiral Analogs - Versatile Reagents in Heterocyclic Chemistry*
Branko Stanovnik **26**
Faculty of Chemistry and Chemical Technology,
University of Ljubljana, Slovenia

11.40-12.10 **Coffee Break**

Plenary Lectures - Part II.

Chairman: **Miha Japelj**

12.10-12.45 *Alternatives to the Present and Creating the Future*
Ante Simonić **27**
Department of Pharmacology, School of Medicine,
University of Rijeka, Croatia

12.45-13.20 *Trends in Cardiovascular Mortality and Risk Factors in the Czech Republic*
Renata Cifková **28**
Institute of Clinical and Experimental Medicine,
Prague, Czech Republic

13.20-15.00 **Lunch Break**

2 Krka Prize Winners' Presentations

Chairman: **Aleš Rotar**

15.00-15.15 *Angiographic Predictors of Arterial Remodeling. An Intravascular Ultrasound (IVUS) Study*
Mariusz Kruk **29**
National Institute of Cardiology, Warsaw, Poland

15.15-15.30 *The Role of Tumor Necrosis Factor a Receptor Type I in Cancer and Rheumatoid Arthritis*
Vesna Galvani **30**
Blood Transfusion Centre of Slovenia, Ljubljana, Slovenia

15.30-15.45 *Preparation and Characterization of Human Recombinant Peptide Fragments of Lactoferrin and CD14 for Binding to Lipopolysaccharides*
Andreja Majerle **31**
National Institute of Chemistry, Ljubljana, Slovenia

15.45-16.00 *Glycopeptide Mimetics as "Puzzles" in Understanding the Biological Phenomena*
Ivanka Jerić **32**
Rudjer Bošković Institute, Zagreb, Croatia

16.00-16.15 *Coordination Compounds with Hyaluronic Acid*
Elizabeta Tratar Pirc **33**
Faculty of Chemistry and Chemical Technology,
University of Ljubljana, Slovenia

16.15-16.30 *Reactivity and Applications of Some Alkyl Arylaminocarbonyldiazene carboxylates*
Roman Lenaršič **34**
KRKA, d. d., Novo mesto, Slovenia

16.30-16.45 *Syntheses and Transformations of Mono and Di(dimethylaminomethylene) Derivatives of 1,3-Acetonedicarboxylates and Related Compounds*
Silvo Zupančič **35**
KRKA, d. d., Novo mesto, Slovenia

16.45-17.10 **Discussion and Final Remarks**

3 Krka Prize Winners' Poster Session

Chairman: **Stanislav Zalar**

Increased Skin Oxygenation after Benzyl Nicotinate Application in Different Liposomes as Measured by EPR Oximetry in vivo
Zrinka Abramović **36**
Faculty of Pharmacy, University of Ljubljana, Slovenia

PROSPECTIVE FOR DEVELOPMENT OF BIOTECHNOLOGY–BASED PHARMACEUTICALS

A. I. Miroshnikov
Shemyakin & Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences, Moscow, Russia

Biotechnology based on the achievements of physical and chemical biology has become one of the few industrial fields, which now determine the level of the economy development virtually in any country in the world. Progress in biological science since 1970 up to the present time allowed biotechnology to take leading position in pharmaceutical industry, with more than 60 % of new-generation therapeutics produced by biotechnological methods.

As an example of the biotech achievements, a pilot-plant technology of recombinant human insulin production has been developed. During the research and development stage of this project, the role of leader peptide and C-peptide of recombinant chimeric proinsulin in formation of intramolecular disulfide bonds has been shown. Specific features of enzymatic transformation of the chimeric proinsulin into fully functional recombinant human insulin have been demonstrated.

The concept of biotechnological production of low-molecular weight hormone oxytocin has been presented. The flow chart of this process starting from the design of a gene encoding chimeric hormone in the form of repetitive lysine-oxytocin sequences, and ending at transformation of the recombinant polyprotein to the native oxytocin will be presented.

Efficient process for production of modified nucleosides has been developed. The presented technology includes chemical synthesis of various heterocyclic bases and enzymatic glycosylation of synthesized nucleoside precursors by recombinant nucleoside phosphorylases isolated from the overproducing strain of *E. coli* purposefully developed for production of this type of enzymes.

1

SURFACE ERODING BIOPOLYMERS FOR DRUG DELIVERY: THERAPEUTIC APPLICATIONS

R. Gurny
School of Pharmacy, University of Geneva, Switzerland

Over the 30 past years, growing interest has been focused on the use of biodegradable polymers for pharmaceutical and biomedical applications, especially in controlled drug delivery, because there is no need to remove the polymer once the drug has been released. Poly(ortho esters) (POE) are hydrophobic and bioerodible polymers that have been investigated for pharmaceutical use since the early 1970s. Among the four described generations of POE, the third (POE III) and fourth (POE IV) are promising viscous and injectable materials which have been investigated in numerous biomedical applications. The most attractive characteristics of these surface-eroding polymers is that drug release rate is constant and predictable. It can be modulated either by changing polymer structure and molecular weight, or by incorporating acidic or basic excipients into the polymeric matrix. POE can be injected by using a conventional syringe; moreover, any kind of drug can be incorporated in the polymeric matrix by simple mixing, without the use of organic solvents.
POE present an excellent biocompatibility, as assessed after intraocular injections in the rabbit eye. It is currently under development for a variety of therapeutic applications, such as ocular delivery for the treatment of glaucoma or vitreoretinal diseases, dental delivery for the treatment of periodontal disease, pain control, veterinary applications and protein delivery. Some preclinical and clinical results show that formulations based on semi-solid POE proved to be promising for numerous pathologies.

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1

TECHNIQUES OF INTERVENTIONAL CARDIOLOGY IN THE XXIST CENTURY

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Percutaneous coronary intervention has been commonly used for the last 20 years. Stents were first implanted for subacute occlusion, then provisional stenting (when necessary) was applied and now direct stenting is an established procedure.
Stents provide mechanical scaffolding decreasing elastic recoil and geometric remodeling, however smooth muscle cell proliferation with neointimal hyperplasia may result in restenosis. Preliminary data indicate that stents coated with immunosuppressive agents with direct slow release substance are able to eliminate restenosis.
Minimizing vessel trauma by direct stenting, applying various coating to increase biocompatibility of stents and prevention of neointimal formation using slow release drugs on stents may revolutionize PCI. Brachytherapy for the prevention and treatment of in-stent restenosis is another option.
For acute MI patients direct PCI with stenting and IIb/IIIa receptor blockers is a common procedure. Platelet IIb/IIIa receptor blockade represents a significant advance in the practice of interventional cardiology and particularly the management of acute coronary syndromes and myocardial infarction. "Facilitated" PCI is the future topic of study as well as the role of potential for combining IIb/IIIa antagonists with thrombolytics and novel inhibitors of thrombin with immediate planned mechanical recanalisation.
Catheter based approach for transendocardial injection of recombinant genes or growth factors to enhance myocardial angiogenesis is a very promising treatment of ischaemic heart disease. Using a magnetic field guided system, electromechanical mapping is performed to localize the sites of LV gene transfer. A steerable catheter with retractable needle can be directed to previously acquired map sites and injections of plasmid DNA encoding vascular endothelial growth factor are made into the viable LV myocardium. This new method enables to revascularise the areas not approachable by PCI or CABG but indications regarding the feasibility, safety and potential efficacy of percutaneous myocardial gene delivery to left ventricular myocardium should be further investigated.
One of the new strategies to improve myocardial revascularization is myogenesis, the guided injection of autologous human myoblasts. Such therapies might be suitable for patients with diabetes, diffuse disease, chronic total occlusions or degenerated saphenous vein grafts.

Challenges for the next 5-10 years are:

- Coated stents for restenosis suppression;
- Adjunctive treatment to improve tissue reperfusion in acute MI;
- Percutaneous myocardial revascularization angiogenesis and myogenesis;
- Distal protection devices;
- Global revascularization;
- New devices for percutaneous closure of atrial and ventricular septal defects.

1

α,β-UNSATURATED ACID DERIVATIVES AND THEIR CHIRAL ANALOGS - VERSATILE REAGENTS IN HETEROCYCLIC CHEMISTRY

B. Stanovnik
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Amino acids and their derivatives are an important class of compounds in organic synthesis, especially as starting materials for the preparation of various heterocyclic systems, such as pyrroles, pyrazoles, imidazoles, 1,2,4-oxadiazoles, pyranones, pyridinones, pyrimidinones and their fused analogs.
In the course of our research interest several groups of reagents have been prepared, such as alkyl α-acylamino-β-(dimethylamino)-α,β-unsaturated amino acids and their derivatives, and chiral cyclic analogs, such as α-(dimethylamino)methylidene derivatives of lactones and lactams.
There are numerous synthetic applications in the synthesis of various heterocyclic systems. In this connection, several methods for the preparation of pyranones, fused pyranones, pyridinones and fused pyridinones, pyrimidinones and fused pyrimidinones, polysubstituted pyrrole-2-carboxylates, 3-aminopyrrole--2-carboxylates and other five and six membered heterocycles, and some natural products and their analogs, such as aplysinopsins, thiaaplysinopsins, azaaplysinopsins and related compounds have been developed.
Several synthetic methodologies have been developed for conversion of chiral 3-(dimethylaminomethylidene)- and 3-cyanomethylidene- substituted γ-lactones and γ-lactams into chiral heteroarylalanines, heteroaryloxohomoalanines, heteroarylalaninols, and other chiral compounds.
Several novel ring transformations will be presented.

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1

ALTERNATIVES TO THE PRESENT AND CREATING THE FUTURE

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We start to understand how our psyche functions, we have almost miraculous power over the physical world, which allows us to think more clearly about making dreams of this kind to come true. This will certainly demand great efforts, a new kind of creative attempt very different from that taught by our present civilisation. Increasingly liberated from the tyranny of denial, we can develop every part of us until we achieve full beauty, reason and humanity, and their freely amalgamated totality will allow us to achieve our full dynamic harmony. We must also learn to accept all the abundant wealth and beauty around us, and taking and choosing from it mould our environment, our relationships and ourselves. Maybe the most suitable attribute for this is "the most noble vision", as Hindemith entitled his book.

The discontent that tortures our soul and body, and which very often results from conflicting and extreme options, is no reason for despair Ð either as individuals or groups. All challenges should be met wisely and practically. We must ask ourselves what we can do within the new playing rules. Yet, we must not to be too modest either, because it is the capacity constantly to discover new rules of the game that allows us to create a new future. To uncover many as yet unknown qualities, we must first understand the world even though this will cause us to lose faith in human exclusiveness, privilege and superiority. This may undermine our narcissism, but should not lessen our curiosity and courage. However, things can be seen differently. Šušnjić writes, "...Life would become intolerable if a person knew what lay ahead. He would become aware of future pain and would suffer in advance so much that he would not enjoy all the good things of the present, because he would be able to see their end. Ignorance is essential to human happiness, and we must admit that people almost always fulfil this condition. We know nothing about ourselves, and absolutely nothing about others. Ignorance fills us with tranquillity, lies make us happy." Although I understand these arguments, I think that ignorance is a much greater source of danger and delusions.

It is obvious that humans are and will be faced with a demanding and long lasting task which, according to Sagan, "...we should accomplish through the significant courage of our questions and the depth of our answers." However, this argument does not free us from responsibility and risk, because it is always easier to ask questions than to answer them.

1

TRENDS IN CARDIOVASCULAR MORTALITY AND RISK FACTORS IN THE CZECH REPUBLIC

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Cardiovascular (CV) disease is the major cause of death in adults and in the elderly in the majority of developed countries, and in many developing countries. Most of the Central and East European countries have very high rates of CV mortality. While there is a downward trend in Northern and Western European countries for the period of 1970-1992, large increases were observed in Central and East European countries. In the Czech Republic, unlike the other post-communist countries, there is a significant decrease in total and CV mortality. The most significant decrease has been achieved in reducing cerebrovascular mortality (both for males and females).

Methods: Three cross-sectional surveys of CV risk factors were conducted within the WHO MONICA Project in six Czech districts in 1985 (n = 2570), 1988 (n = 2768), and 1992 (n = 2342). In 1997/98, another population survey (a 1% random sample, aged 25-64, mean age 45 years) was performed in the six original districts (n = 1990) and in three other districts (n = 1219). The respondence rate was 64.4%.

Results: Over a 12-year period, there was a statistically significant decrease in the prevalence of smoking in males (from 49.2% to 36.8%, $p < 0.001$) and no change in smoking habits in females. Body mass index did not change in either sex, and obesity is still highly prevalent in the Czech population, especially aged 45+. A significant reduction, both in systolic (from 133.6 ± 20.2 to 127.8 ± 17.5 mmHg; $p < 0.001$) and diastolic blood pressure (from 84.1 ± 11.3 to 81.5 ± 10.1 mmHg; $p < 0.001$), was achieved. There was also a decreased prevalence and improved control of hypertension. A remarkable drop in total cholesterol was seen in both sexes (males: from 6.21 ± 1.29 to 5.65 ± 1.15 mmol/L; $p < 0.001$; females: from 6.18 ± 1.26 to 5.53 ± 1.21 mmol/L; $p < 0.001$). The 1997/98 survey showed a better lipid profile and lower diastolic blood pressure in the six original MONICA (Multinational MONItoring of trends and determinants in CArdiovascular disease) districts.

Conclusions: The remarkable improvement in CV risk factors documented in a 12-year follow-up period may contribute to the decrease in CV and total mortality, contrary to the trend in the other post-communist countries.

ANGIOGRAPHIC PREDICTORS OF ARTERIAL REMODELING. AN INTRAVASCULAR ULTRASOUND (IVUS) STUDY

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Recognition of coronary artery plaque remodeling provides substantial clinical information as preexisting expansion remodeling as seen on IVUS is linked to an increased plaque vulnerability - the underlying cause of acute coronary syndromes and to restenosis following coronary interventions. Routine coronary angiography does not reveal the remodeling process. It may be well assessed in vivo by IVUS however this examination is practical on routine basis. Angiographic surrogate of plaque vulnerability is its complex morphology.
Moreover, presence of systemic and genetic factors involved into development of atherosclerosis suggests that plaque vulnerability may be a disseminated phenomenon.
Therefore, we combined coronary angiography and IVUS to determine whether plaque vulnerability is a disseminated phenomenon and whether remodeling status of the target lesion may be predicted by coronary angiography findings.

Methods: Preinterventional IVUS pull back maneuver was performed in 55 patients (37 males, 18 females, age 55.6±10.7). The remodeling index (RI) was defined as vessel area at the target lesion site divided by that of average reference segments. The RI ≥1.05 was assumed as positive remodeling (+veR). Coronary angiograms were analyzed and all plaques ≥30% stenosis were identified and classified into complex or smooth groups. Complex plaque was defined if multiple irregularities, overhanging edges, ulcerations, total occlusion or thrombus were present. The remaining lesions accounted smooth. Multiple complex lesions were defined as ≥2 complex plaques. Clinical, angiographic and IVUS continuous variables for the 2 groups were compared with an unpaired Student's *t* test. Categorical variables were compared with the chi-square test. To identify independent angiographic predictors of positive remodeling at the target lesion site multivariate backward stepwise logistic regression analysis was applied. In this model presence of: multiple lesions, complex target lesions, any complex plaques, any multiple complex plaques were analyzed.

Results: +veR was found in 23 of target lesions examined by IVUS. Angiographically complex plaques were present in 12 target lesions and in 58 out of all 163 analyzed plaques. For the two groups of patients (with positive remodeling versus no positive remodeling), there was no significant difference in the mean number of lesions (3.1±1.3 vs 2.8±1.1; p ns) whereas both the mean number of angiographicaly complex lesions and the mean number of angiographicaly complex lesions independent of the target were more frequent on the group with positive remodeling at the target site as seen on IVUS (1.6±1.3 versus 0.7±1.0; p=0.007 and 1.2±1.2 versus 0.6±0.9; p=0.049 respectively).

Multiple logistic regression analysis revealed that the strongest independent angiographic predictor of +veR at the target site was the presence of multiple complex plaques (p=0.015).

Conclusions: Our results support hypothesis that plaque vulnerability, if present, may be a disseminated phenomenon. Moreover, coronary angiography is able to provide information regarding remodeling status of the target site.

2

THE ROLE OF TUMOR NECROSIS FACTOR α RECEPTOR TYPE 1 IN CANCER AND RHEUMATOID ARTHRITIS

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The importance of the tumor necrosis factor alpha (TNFα) is well determined in connection with malignant diseases and autoimmune diseases. On the other hand, the importance of cytokine receptors is not well known. TNFα is a member of a cytokine family and is produced by stimulated mononuclear cells and some other cells. In accordance with the production of TNFα, the two TNF receptors, p55 and p75, are produced. Both receptors can be found in human serum or urine in soluble form and are supposed to be the natural inhibitors of the TNFα. Soluble receptors are cleavage products of the membrane bound receptors and might compete with them for the free TNFα.

Monoclonal antibodies against p55 were prepared and sensitive ELISA was developed. We found out that the concentration of soluble receptor p55 is considerably higher in of patients with malignant melanoma, compared to serum levels of p55 in a healthy population. We were also able to distinguish between patients with malignant melanoma who responded to the chemotherapy and those who did not respond to the same therapy. We therefore presume that monitoring the concentration of p55 in patients' serum would be a good prognostic factor of the progression of the disease and may be of additional help to the clinicians. According to our study, we were able to support the hypothesis that more aggresive malignant cells of metastasis expressed significantly more p55 mRNA and therefore more soluble p55 than primary tumors or/and healthy tissue cells. We presume that these findings might suggest that the soluble p55 contributes to the tumor formation and survival by inhibiting immunological mechanisms *in vivo*. We also found that concentration follow-up of soluble p55 and TNFα in serum of patients with rheumatoid arthritis might be a valuable marker for the outcome of the treatment with leflunomide and methotrexate.

2

PREPARATION AND CHARACTERIZATION OF HUMAN RECOMBINANT PEPTIDE FRAGMENTS OF LACTOFERRIN AND CD14 FOR BINDING TO LIPOPOLYSACCHARIDES

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We have expressed functional fragments of human CD14, the high affinity cellular receptor for lipopolysaccharides (LPS), in bacteria *Escherichia coli* and yeast *Pichia pastoris*. Recombinant CD14 from both bacteria and yeast bind to LPS (1, 2). In bacteria *Escherichia coli* we have produced recombinant peptide LF12 in the form of fusion protein. LF12 is based on eleven amino acids of human lactoferrin, which contributes to the high-affinity binding to LPS and to antimicrobial activity against Gram-negative and Gram-positive bacteria (3). We have investigated the role of lipophylicity in a series of lipopeptides based on peptide LF12 on antimicrobial and LPS neutralizing activity. Result of modification of parent peptide with lipid chains of various length and saturation was enhancement of LPS neutralizing and antimicrobial activity. Since lipopeptides have shown activity comparable to antibiotics, which are used in medicine today, we have taken out a patent for our invention (4).

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2

GLYCOPEPTIDE MIMETICS AS "PUZZLES" IN UNDERSTANDING THE BIOLOGICAL PHENOMENA

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A diversity and complexity of naturally occurring glycoproteins demand well-defined glycopeptide mimetics to probe the nature of biological processes. Among a number of approaches, we choose to study phenomena associated with a complex group of reactions, collectively known as the Maillard reaction. Initial non-enzymatic reaction between reducing sugars and amino groups of peptides or proteins induces a whole cascade of chemical transformations ending with advanced glycation end products (AGEs). AGEs are reactive species easily trapped by proteins, causing physical, chemical and functional changes and as a long-term consequence, irreversible protein damages. It is know today, that AGEs are involved in diabetic and aging processes, as well as Alzheimer's and vascular diseases.
In our study monosaccharide esters of peptides were used as tools to light up some of the open issues, namely the reactivity of such sugar-peptide esters and especially the role of the length and composition of the peptide part in inter- and intramolecular reactions. Besides, interplay of chemical synthesis, NMR spectroscopy and mass spectrometry allow obtained results to be considered as "puzzles" necessary for the understanding of more complex "images".

2

COORDINATION COMPOUNDS WITH HYALURONIC ACID

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Supervisor: P. Bukovec
Faculty of Chemistry and Chemical Technology, University of Ljubljana, Slovenia

Hyaluronic acid is a glycosamonoglycan and consists of repeating disaccahride units composed of 2-acetamido--2-deoxy-D-glucose and D-glucuronic acid linked by alternating β(1-3) and β(1-4) glycosidic bonds. The number of disaccharide units varies between 250 and 25000.
Biological activity of hyaluronate molecule strongly depends on its conformation. Binding cations to a macromolecule has the major effect on conformation. In this research my attention was focused on interaction of hyaluronate molecule with manganese(II), nickel(II), cobalt(II), copper(II), and zinc(II) ions. Solid-state products were prepared from slightly acid water hyaluronate solutions with precipitation with cold ethanol. All products are amorphous and soluble only in water. The compounds obtained in this manner were characterised by employing magnetic measurements, elemental analysis, IR spectroscopy, TG analysis, and EXAFS measurements. In metal hyaluronates with general formula $M(C_{14}H_{20}O_{11}N)_2 \times H_2O$ ($M = Mn^{2+}$, Ni^{2+}, Co^{2+}, Cu^{2+}, Zn^{2+}) the metal ion is bound to the two repeating disaccharides units across carboxylate groups in glucuronic acid. The metal ions are coordinated with oxygen atoms that belong to carboxylates groups and water molecules.
Molecular modelling (QM/MM method) was used for calculating the energetically most favourable distances between copper(II) ion and oxygen atoms in copper(II) hyaluronate. The results were compared with distances obtained by EXAFS measurements. The calculations have shown the coordination polyhedron, surrounding the copper(II) ion, is disordered octahedron. The calculated distances between Cu and O atoms are in good agreement with experimental distances.

2

REACTIVITY AND APPLICATIONS OF SOME ALKYL ARYLAMINO CARBONYLDIAZENECARBOXYLATES

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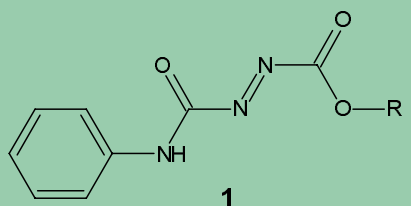
Alkyl arylaminocarbonyldiazenecarboxylates (diazenes **1**) contain several reactive centres: an aromatic ring, the amide group, an electrophilic nitrogen of the azo group and the ester group.
Their reactivity was first studied in aromatic electrophilic substitutions. $ZrCl_4$ was selected as a catalyst. Intermolecular reactions took place in the presence of activated aromates, in their absence reactions proceeded intramoleculary. Aromatic substitutions were regioselective.
The second type of reactions involved electrophilic substitutions of 1,3-dicarbonyl compounds. In the case of $ZrCl_4$ catalysed reactions 2-imidazolones were isolated. With intention to explore basic catalysed substitutions DBN was employed and led to the formation of 2-imidazolidinones. Compounds that were formed under basic conditions were transformed in acidic media to 2-imidazolones which were the same as those obtained in $ZrCl_4$ catalysed reaction.
We also studied transformations of diazenecarboxylates into diazenedicarboxamides as the latter compounds were found to exhibit biological activity.



1

2

SYNTHESES AND TRANSFORMATIONS OF MONO AND DI(DIMETHYLAMINOMETHYLENE) DERIVATIVES OF 1,3-ACETONEDICARBOXYLATES AND RELATED COMPOUNDS

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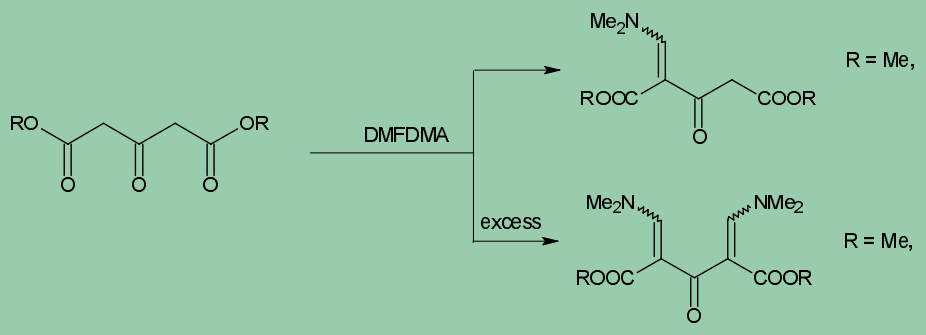
Dimethyl and diethyl 1,3-acetonedicarboxylate and 1,5-diphenyl-1,3,5-pentanetrione as compounds with two activated methylene groups were transformed with DMFDMA into dimethyl and diethyl 1-dimethylamino-3-oxobut-1-ene-2,4-dicarboxylate, dimethyl and diethyl 1,5-bis(dimethylamino)--3-oxopenta-1,4-diene-2,4-dicarboxylate and 1,5-diphenyl-2,4-bis(dimethylaminomethylene)penthane--1,3,5-trione. Dimethyl and diethyl 1-dimethylamino-3-oxobut-1-ene-2,4-dicarboxylate were employed in the synthesis of: pyridones, pyrazoles, pyrimidines, pyridopyrimidines, pyranones and related systems.
On the other hand dimethyl and diethyl 1,5-bis(dimethylamino)-3-oxopenta-1,4-diene-2,4-dicarboxylate and 1,5-diphenyl-2,4-bis(dimethylaminomethylene)penthane-1,3,5-trione were transformed into symmetrically substituted 4-pyridones.



POVEČANJE OKSIGENACIJE KOŽE PO APLIKACIJI BENZIL NIKOTINATA V LIPOSOMIH, IZMERJENO Z EPR METODO *IN VIVO*

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Povečana oksigenacija kože deluje pozitivno na zdravljenje revmatičnih obolenj, pospeši hitrost celjenja poškodovane kože in poveča učinkovitost radioterapije kožnega raka. *In vivo* proučevanje perkutane absorpcije estrov nikotinske kisline, ki posredno s povečanjem prekrvavitve kože povzročijo dvig parcialnega tlaka kisika v koži, lahko kvantitativno izmerimo z elektronsko paramagnetno resonanco (EPR).
V raziskovalni nalogi smo benzil nikotinat vgradili v liposome z različno sestavo ter s pomočjo nizkofrekvenčne EPR neposredno spremljali časovno odvisnost povečanja pO_2 v koži mišk po topikalni aplikaciji liposomov v hidrogelu. Učinek nikotinata vgrajenega v liposome smo ovrednotili glede na čas, potreben za začetek delovanja, čas ko pO_2 doseže maksimum in učinkovitost absorpcije, izraženo s površino pod krivuljo. Eksperimentalno smo dokazali, da je absorpcija benzil nikotinata vgrajenega v liposome učinkovitejša kot absorpcija iz samega hidrogela. Učinkovitost penetracije nikotinata skozi kožo je odvisna od sestave in tudi velikosti liposomov, ki pomembno vplivata na hitrost absorpcije, manj pa na maksimalno povečanje pO_2. Značilno se poveča pO_2 v koži po večkratni aplikaciji.
Zaključimo lahko, da je elektronska paramagnetna resonanca primerna metoda za *in vivo* spremljanje povečanja oksigenacije kože. Rezultati tovrstnih meritev lahko uspešno prispevajo pri načrtovanju hiperemikov.

INCREASED SKIN OXYGENATION AFTER BENZYL NICOTINATE APPLICATION IN DIFFERENT LIPOSOMES AS MEASURED BY EPR OXIMETRY *IN VIVO*

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Improved skin oxygenation has a positive effect on the treatment of rheumatism, it stimulates the healing process of damaged skin and increases the efficiency of radiotherapy in skin cancer treatment. The penetration of nicotinate esters through the skin which provoke increase in blood flow and level of oxygen in skin, can be measured by electron paramagnetic resonance (EPR) *in vivo*.
For the purposes of this research, benzyl nicotinat (BN) was incorporated in liposomes with different composition. Following the topical application of liposomes in hydrophilic gel onto the skin of the mice, pO_2 increase was measured by means of low-frequency EPR. The effect of nicotinate was evaluated in three different aspects: lag-time, time needed for the maximum increase in pO_2 and overall efficiency expressed by the area under curve. We have proved the absorption of BN incorporated in liposomes to be more effective than the absorption from hydrophilic gel. The efficiency of nicotinate penetration through the skin depends on the composition and size of liposomes, which significantly effect the time when BN starts to act, less so the maximum increase of pO_2 in skin. After repeated application of liposomes the increase of pO_2 was noticed.
The EPR oximetry is a suitable method for *in vivo* observation of skin oxygenation increase. The findings of this study indicate the potential use in development of topical preparations with hyperemics.

3

RAZGRADLJIVOST ORGANSKIH SNOVI V ODPADNI VODI FARMACEVTSKE INDUSTRIJE

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Določevanje biorazgradljivosti organskih snovi v odpadni vodi ima velik pomen pri oceni njihovih vplivov v naravnem vodnem okolju. Biološka razgradnja pomeni pretvorbo organske snovi v neko drugo obliko. Slovenska zakonodaja predpisuje za določevanje biološke razgradljivosti odpadnih vod Zahn-Wellensov test (ISO 9888). Zahn-Wellensov test je preprost test s predhodno spranim aktivnim blatom. Testna mešanica je mešana na magnetnih mešalnikih in prezračevana. Razgradnji sledimo z analizo kemijske potrebe po kisiku (KPK). Namen te raziskave je ocena biološke razgradljivosti specifične odpadne vode farmacevtske industrije (vzorec E) in določenega proizvoda iste industrije (vzorec P) v sedmih dneh. Za kontrolo primernosti testnih pogojev je bila spremljana razgradnja anilina. Začetna KPK koncentracija obeh vzorcev in anilina je bila približno 400 mg/l. Koncentracija aktivnega blata je znašala 0,6 g/l. Ugotovljena je bila izrazita razlika v biološki razgradljivosti teh dveh vzorcev. Vzorec E se je razgradil skoraj popolnoma (92%) v sedmih dneh. Pri vzorcu P pa razgradnja ni dosegla tako visoke stopnje, saj je tudi po desetih oz. petnajstih dneh znašala le 59%. Anilin je lahko razgradljiva organska snov, ki se je v sedmih dneh razgradila 89% in v skladu s pričakovanji.

DEGRADABILITY OF ORGANIC COMPOUNDS IN WASTEWATER OF PHARMACEUTICAL INDUSTRY

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Determination of biodegradability of organic compounds in waste water has great importance in the assessment of their influences in the natural water environment. Biological degradation means transformation of an organic matter into another form. Slovene legislation prescribes for the determination of biodegradability of waste water the Zahn-Wellens test (ISO 9888). It is a simple batch test with prewashed activated sludge. The test mixture is mixed with magnetic stirrers and aired. The degradation is monitored by analysis of chemical oxygen demand (COD). The purpose of this investigation was the assessment of biodegradability of specific waste water from pharmaceutical industry (sample E) and of a certain product of the same industry (sample P) within seven days. For the control of suitability of test conditions the degradation of aniline was observed. The initial COD concentration of both samples and aniline was about 400 mg/l. The concentration of activated sludge was 0,6 g/l. A significant difference was observed in the biodegradability of these two samples. Sample E was degraded almost completely (92%) within seven days. In sample P degradation did not reach such a high level, even after ten and fifteen days resp. it reached only 59%. Aniline is an easily degradable matter, which was degraded almost completely (89%) within seven days - as expected.

3

SINTEZA AMFIFILNIH NITROKSIDOV ZA PROUČEVANJE NANO-DELCEV Z EPR METODO

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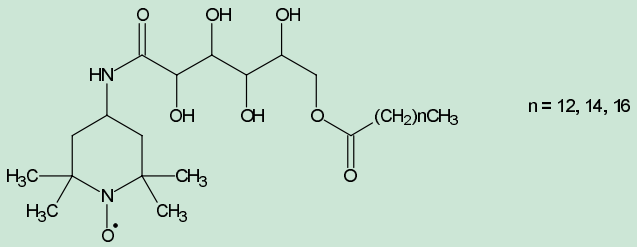
Nanodelci so nova, zelo perspektivna farmacevtska oblika za ciljano dostavljanje zdravilnih učinkovin. Za proučevanje vgrajevanja modelnih spojin v nanodelce in študij njihovih lastnosti in njihovo potencialno sledenje v *in vivo* sistemu se je elektronska spinska resonanca (EPR) izkazala kot zelo uporabna metoda.
Sintetizirali smo amfifilne, neionske spinske označevalce, s katerimi lahko nanodelce ustrezno spinsko označimo. Hidrofilni del molekule sestavljata nitroksilni radikal in sladkorna enota, lipofilni del pa predstavlja višja maščobna kislina. V prvem delu smo ceneno izhodno spojino 4-amino-2,2,6,6-tetrametilpiperidin selektivno zaščitili na 4-amino mestu s karbamatno skupino. Sekundarno amino skupino smo nato oksidirali v nitroksilni radikal z vodikovim peroksidom in natrijevim volframatom kot katalizatorjem. Po odstranitvi karbamatne zaščite z bazično hidrolizo smo molekuli z reakcijo aminolize δ-laktona-D-glukonske kisline pripeli hidrofilni del. Nastalo spojino smo v drugem delu selektivno acilirali z različnimi kislinskimi kloridi višjih maščobnih kislin. Modelna spojina je predstavljena na spodnji sliki:



S preprosto EPR meritvijo modelne spojine v različnih topilih in mleku smo dokazali njeno uporabnost kot spinskega označevalca nanodelcev in/ali liposomov, saj so iz spektrov razvidne njene amfifilne lastnosti.

SYNTHESIS OF AMPHYPHILIC NITROXIDES FOR ANALYSIS OF NANO-PARTICLES WITH ESR METHOD

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Solid lipid nanoparticles (SLN) are new, perspective dosage form for targeted delivery of drugs. Electronic spin resonance (ESR) has proven to be a useful method for studying incorporation of spin-labeled compounds into SLN, properties of spin-labeled SLN and their potential tracing *in vivo*.
We have synthesized amphiphylic, nonionic spin labels, which can be used for labeling SLN as well as model membranes such as liposome. Hydrophilic moiety of model compound is composed of a nitroxyl radical, tetrahydroxy spacer group and a proper lipophilic propertiy is achieved by means of incorporating fatty acid residue into the molecule. 4-Amino-2,2,6,6-tetramethylpiperidine-1-oxyl prepared from corresponding 4-amino-2,2,6,6-tetramethylpiperidine reacted with δ-lactone of D-gluconic acid. After than the primary hydroxyl group have been selectively acylated by various fatty acid chlorides.
A simple EPR study of our model compound in different solvents and milk has proven that these types of compounds can be used for labeling SLN and/or liposomes.

3

VPLIV JASMONSKE KISLINE NA VSEBNOST TAKSANOV V CELIČNI SUSPENZIJSKI KULTURI TISE (*TAXUS X MEDIA* REHD.)

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Taksani so rastlinski sekundarni metaboliti, med njimi je najbolj znan taksol, ki ga uporabljajo za zdravljenje nekaterih vrst raka. Klasično taksol pridobivajo iz lubja tise *Taxus brevifolia* in s semisintezo iz prekurzorjev. Tkivne kulture tise predstavljajo alternativni vir taksola in drugih taksanov. Jasmonati so vključeni v odgovor rastline na patogene in zato lahko delujejo kot elicitorji sekundarnega metabolizma.
Namen dela je bil preko kalic in kalusa vzpostaviti in optimizirati celično suspenzijsko kulturo tise ter pri izbrani celični liniji ugotoviti vpliv jasmonske kisline (JA) na rast kulture in vsebnost taksanov.
Vzpostavili smo uspešen postopek *in vitro* kalitve semen tise. Nastale kalice smo uporabili za vzpostavitev kulture kalusa. Najboljši vir izsečkov za iniciacijo kalusa so bili stebla in korenine, najboljše gojišče pa je vsebovalo hormona 2,4-D in kinetin. Vzpostavili smo več linij celičnih suspenzijskih kultur tise, ki so se med seboj razlikovale tako po rasti kot po vsebnosti taksanov.
Pri liniji vrste *Taxus* x *media* smo ugotavljali vpliv JA (100 µM, dodana 7. dan po zadnji subkultivaciji) na rast in vsebnost taksanov (skupni taksani, 10-deacetil bakatin III, bakatin III, cefalomanin in taksol). Slednje smo določili z encimsko imunskim testom (ELISA) in HPLC. JA je nekoliko inhibirala rast (določeno s svežo in suho maso) ter statistično značilno povečala vsebnost vseh taksanov tako v celicah kot v gojišču. Časovna dinamika akumulacije opazovanih taksanov v celicah in gojišču se po elicitaciji z JA ni spremenila. Največja vsebnost taksola je bila v celicah 182 µg/g suhe mase (26. dan po subkultivaciji) in v gojišču 6,05 mg/l (20. dan).

THE EFFECT OF JASMONIC ACID ON TAXANE CONTENT IN CELL SUSPENSION CULTURE OF YEW (*TAXUS X MEDIA* REHD.)

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Taxanes are plant secondary metabolites. Among them, taxol is used for the treatment of some forms of cancer. Traditionally, it is extracted from the bark of *Taxus brevifolia* or produced semi-synthetically from precursors. Yew tissue culture is an alternative source of taxanes. Jasmonates are involved in the plant's defence response against pathogens and may therefore act as successful elicitors of secondary metabolism.
The object of this work was to establish yew cell suspension culture, via embryo and callus culture, to optimise it, and to determine the effect of jasmonic acid (JA) on growth and taxane content in a selected cell line.
A successful protocol for *in vitro* germination of yew seeds was developed. The seedlings formed were used to establish the callus culture. The best sources of explant for initiation and growth of callus were stems and roots from the seedlings, and the best medium contained plant hormones 2,4-D and kinetin. Several lines of cell suspension cultures, which varied in growth and taxane content, were established.
In a selected cell line of yew *Taxus* x *media*, the effect of JA (100 µM, added 7 days after the last subculture) on growth and production of total taxanes, 10-deacetylbaccatin III, baccatin III, cephalomanine and taxol was determined. Taxanes were determined and quantified by ELISA and HPLC. JA slightly inhibited growth (determined by fresh and dry weight) and significantly increased taxane content in cells and in the growth medium. The time course of the taxane accumulation was not changed after JA elicitation. The highest taxol content in the cells and was 182 µg/g dry weight and in the medium 6,05 mg/l, measured 26 and 20 days respectively, after subculture.

3

TRŽENJE ZDRAVIL BREZ RECEPTA

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Pod pojmom samozdravljenje razumemo dejanja, s katerimi si vsak posameznik skuša pomagati v primeru bolezni ali slabega počutja, oziroma deluje preventivno v smislu ohranjanja lastnega zdravja in dobrega počutja. Izvaja se s pomočjo zdravil, ki so posamezniku dosegljiva brez recepta, z zelišči ali s pomočjo alternativne medicine.
Diplomsko delo sva razdelili na tri sklope. V prvem delu sva govorili o osnovnih trženjskih konceptih, ki so pomembni za razumevanje samega pojma trženje. Predstavili sva tudi faze razvoja marketinške znanosti, in sicer od prvotnega marketinga (menjava blago za blago), preko "agresivnega" marketinga, se pravi prodajne usmeritve podjetij, kjer je šlo za vsiljevanje izdelkov potrošnikom; nadaljevali sva s fazo marketinškega upravljanja, kjer se podjetja že začno zavedati pomena svojih potrošnikov, njihove želje in potrebe pa skušajo čimbolj zadovoljiti. Zadnjo fazo v razvoju pa predstavlja t.i. celostni marketing, ki sestoji iz treh ravni ekvivalentne menjave (marketing z zaposlenimi, menjava z déležniki ter menjava z družbenim in naravnim okoljem podjetja).
V drugem delu sva se osredotočili na zdravila za samozdravljenje, to je njihovo uporabo, prodajo na tujih trgih, obdelali pa sva tudi evropsko zakonodajo na področju samozdravljenja. Z moralnega in etičnega vidika je področje zdravil zelo občutljiva tema. Preučili sva zakonodajo, ki regulira pojavnost in oglaševanje zdravil v različnih medijih in le-to primerjali s tujimi državami in vse to aplicirali na konkreten primer, to je farmacevtsko podjetje Krka.
V tretjem delu sva s pomočjo anketnega vprašalnika skušali izvedeti, kakšen je odnos Slovencev do samozdravljenja. Anketo sva izvedli v petih slovenskih regijah, anketirali sva 100 ljudi, kar sicer pomeni, da je bil vzorec anketiranih premajhen, da bi bil reprezentativen, kljub temu pa sva mnenja, da najine ugotovitve prijetno popestrijo vsebinsko zasnovo najine naloge.

MARKETING OF OTC DRUGS

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Self-medicaton is defined as an act by which people have the right and duty to participate actively, individually and in groups, at the planning and realisation of their own health care. People use the medicaments intended for self-medication in case of feeling bad or just to preserve their own good health. It is carried out by OTC drugs (non prescription drugs), medical herbs or alternative medicine.
In the first part of our diploma work basic marketing concepts important for further understanding are discussed. Different phases of marketing development are also presented (basic marketing, aggressive marketing, classical marketing and holistic view of marketing). In second part self-medication drugs, especially their use, sale in foreign markets and legislation from this area are presented. Medicinal field of work is known as very delicate, considering moral and ethical point of view. The study of the legislation of drug advertising in Slovenia has been performed and compared to the situation in foreign countries. In the third part of the study the public opinion of self-medication has been evaluated using a questionnaire. The research was carried out on the sample of 100 individuals from 5 Slovenian regions.

3

OBVLADOVANJE ČRTNIH KOD Z UPORABO BAZ PODATKOV V KRKI

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Naloga predstavlja sistem črtnih EAN-13 in Farma kod za zdravila v podjetju Krka, tovarna zdravil, d. d. Opisani so vzroki za uvedbo kod ter uporabljene kode. Podrobno je opisan sistem vnosa v bazo podatkov, način ažuriranja in izpisa kod.
Ugotovila sem naslednje prednosti uvedbe sistema črtnih kod: večjo konkurenčnost podjetja, zanesljivejšo kontrolo izdelkov in širšo dostopnost do podatkov o kodah izdelkov. Med slabostmi sistema kodiranja v podjetju sem opazila parcialno obravnavanje sistema in pomanjkljivo organiziranost vnosa kod. Priložnosti, ki jih podjetje lahko v prihodnosti izkoristi za izboljšanje svojega poslovanja, so: uvedba UCC/EAN-128 kod namesto EAN-128 kod, računalniška izmenjava podatkov in vpeljava standardne kode zabojnika. Pasti, v katere smo se pri razvijanju obstoječega sistema ujeli, pa vidim v premajhnem številu razpoložljivih Farma kod in problemih v zvezi z neveljavnimi šiframi končnih izdelkov in neveljavnimi šiframi embalažnih materialov.
V nalogi so nakazani predlogi za izboljšanje obstoječega sistema in za nadaljnji razvoj sistema v podjetju.

BAR CODES MANAGEMENT USING DATABASES IN KRKA

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This work presents the system of EAN-13 and Pharma-Code bar codes for drugs in the company Krka, d. d. The causes for introduction and use of bar codes are described. The system of data import to central database, rearangement of data and display of codes are presented in details.
The next advantages of used bar code systems have been identified: greater competitive position of the company, more reliable control of products and better accessibility of product bar code data. Some weakness of the used system have been found: partial treating of coding system in the company and weak organisation of bar code data input. The opportunities for the improvement of the business operations are: replacement of EAN-128 with UCC/EAN-128 codes, introduction of EDI and introduction of SSCC. Traps we were caught in were: insufficient number of available Pharma-Codes, problems connected with invalid product and invalid packaging material code numbers .
Improvement of existent system of bar codes and the further development of the system in the company are proposed.

3

VPLIV DEKSAMETAZONA NA IZRAŽANJE LANOSTEROLNE 14α–DEMETILAZE V ČLOVEŠKIH CELIČNIH LINIJAH

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V diplomski nalogi smo želeli ugotoviti, ali sintezni glukokortikosteroid deksametazon vpliva na izražanje lanosterolne 14α-demetilaze v pogojih *ex vivo* in ali v tem mehanizmu uravnavanja izražanja sodeluje tudi pregnanski X receptor. Lanosterolna 14α-demetilaza (CYP51) je eden ključnih encimov pri biosintezi holesterola. Pregnanski X receptor sodi med novejše jedrne receptorje sirote, ki nimajo natančno določenega endogenega liganda, temveč je široko specifičen.
V nesmrtne človeške celice smo vnesli sporočilni plazmid D7-CAT, ki je sestavljen iz gena za kloramfenikol acetil transferazo (CAT) pod kontrolo promotorja človeške lanosterolne 14α-demetilaze. V nekatere celice smo vnesli še plazmid, ki omogoča prekomerno izražanje človeškega pregnanskega X receptorja. Po transfekciji smo celice gojili v gojišču brez ali z dodatkom deksametazona. Analiza aktivnosti encima CAT nam je povedala, kako sta deksametazon in pregnanski X receptor vplivala na aktivacijo promotorja gena CYP51.
Deksametazon štirikrat poveča izražanje lanosterolne 14α-demetilaze preko do sedaj še neznanega mehanizma (ali mehanizmov) uravnavanja izražanja, v katerem pa ne sodeluje pregnanski X receptor. 5,6-kratno aktivacijo promotorja lanosterolne 14α-demetilaze pa smo dobili pri kotransfekciji celic s plazmidom, ki omogoča prekomerno izražanje pregnanskega X receptorja, in ob dodatku deksametazona. Dokazali smo, da deksametazon vpliva na endogeni metabolizem holesterola. Čeprav je statistično nismo uspeli dokazati, obstaja možnost, da izražanje lanosterolne 14α-demetilaze aktivira tudi pregnanski X receptor, katerega ligandi so številne zdravilne učinkovine.

THE ROLE OF DEXAMETHASONE IN EXPRESSION OF LANOSTEROL 14α–DEMETHYLASE IN HUMAN CELL LINES

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The thesis attempts to establish whether the synthetic glucocorticosteroid dexamethasone effects the expression of lanosterol 14α-demethylase in *ex vivo* conditions, and whether the pregnane X receptor participates in this regulation. Lanosterol 14α-demethylase (CYP51) is one of the crucial enzymes of cholesterol biosynthesis. The pregnane X receptor is one of the recently discovered orphan nuclear receptors without a known endogenous ligand.
Reporter D7-CAT plasmid, which consists of a gene for chloramphenicol acetyl transferase (CAT) controlled by the promoter of human lanosterol 14α-demethylase, was transfected into immortal human cells. Some cells were additionally co-transfected with a plasmid, that enables overexpression of the human pregnane X receptor. After transfection, cells were grown in a culture medium with or without the addition of dexamethasone. The analysis of CAT activity showed the effects of dexamethasone and the pregnane X receptor on CYP51 promotor activation.
Dexamethasone increases the expression of lanosterol 14α-demethylase four times via an as-yet unknown regulatory mechanism (or mechanisms) where pregnane X receptor is not involved. On the other hand, 5,6-fold activation of the promoter was established after cotransfection of cells with pregnane X receptor overexpression plasmid and the addition of dexamethasone. This work shows that dexamethasone affects the endogenous metabolism of cholesterol. Although we were not able to prove this statistically, a possibility exists that the expression of lanosterol 14α-demethylase is also activated by pregnane X receptor, whose ligands are numerous drugs.

3

KORONARNA BOLEZEN V POVEZAVI S HIPERLIPOPROTEINEMIJO S KLINIČNEGA, EPIDEMIOLOŠKEGA IN FAMAKOEKONOMSKEGA VIDIKA

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Namen moje diplomske naloge je bil prikazati koronarno bolezen s kliničnega, epidemiološkega in farmakoekonomskega vidika. Osredotočila sem se na hiperlipoproteinemijo kot enega izmed glavnih dejavnikov tveganja koronarne bolezni in na zdravljenje le-te z zaviralci HMG-CoA reduktaze.
Z metodo metaanalize sem dokazala učinkovitost statinov pri preprečevanju koronarnega dogodka, smrti zaradi koronarnega vzroka, kardiovaskularnega vzroka in pri zmanjšanju skupne umrljivosti. S pomočjo raziskave CINDI sem ocenila prevalenco hiperlipidemije v Sloveniji in delež bolnikov, ki ustreza evropskim in slovenskim kriterijem za zdravljenje s hipolipemiki. S farmakoekonomsko analizo sem želela oceniti povprečne letne stroške, ki bi nastali zaradi zdravljenja hiperlipidemije vseh Slovencev, ki ustrezajo kriterijem za zdravljenje s hipolipemiki. Preventivno optimalno zdravljenje teh prebivalcev z Vasilipom® bi letno znašalo 2,25% BDP. Predstavila sem povprečne letne stroške vseh načinov zdravljenja in preventive koronarne bolezni na posameznika in izračunala razliko med stroški, ki bi nastali v petih letih v primeru nezdravljenja hiperlipidemije in v primeru preventivnega zdravljenja z Vasilipom® vseh prebivalcev, ki bi drugače v enakem obdobju doživeli akutni miokardni infarkt in/ali potrebovali revaskularizacijski poseg. Na koncu pa sem naredila še projekcijo umrljivosti zaradi koronarnega in kardiovaskularnega vzroka prebivalcev Slovenije s hiperlipidemijo, ki bi potrebovali zdravljenje s hipolipemiki, za obdobje desetih let v primeru preventivnega zdravljenja z simvastatinom in nezdravljenja hiperlipidemije. Preventivno zdravljenje hiperlipidemije s statini je stroškovno najučinkovitejše in najkoristnejše v primerjavi z ostalimi načini zdravljenja koronarne bolezni.

CORONARY HEART DISEASE WITH HYPERLIPOPROTEINEMIA FROM CLINICAL, EPIDEMIOLOGICAL AND PHARMACO-ECONOMICAL POINT OF VIEW

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The purpose of my research was to evaluate coronary heart disease from clinical, epidemiological and pharmacoeconomical point of view. Inhibitors of HMG-CoA reduktaze are first-line drugs intended for treatment of hyperlipoproteinemia, presenting the main risk factor for coronary heart disease.
With metaanalysis I proved efficacy of statins in preventing coronary events, mortality resulting from coronary and cardiovascular events and its efficiency in lowering total mortality. On the basis of the CINDI research I assessed the prevalence of hyperlipoproteinemia in Slovenia and the share of Slovenians that would need a treatment according to the European and Slovenian guidelines. With pharmacoeconomical analysis I assessed an average year's costs, if all Slovenians who need treatment with hypolipemics would be treated with Vasilip®. Optimal preventive treatment of those Slovenians with Vasilip® would represent 2,25% BDP. Represented are also average year's costs of other methods intended for the treatment of coronary heart disease per person. I assessed 5-years difference between costs of non-treatment of hyperlipidemia and preventive treatment with Vasilip® of patients who would in the same period suffer from an acute myocardial infarction and/or revasculating procedures. I made a 10-year's projection of mortality due to the coronary and cardiovascular causes of Slovenians suffering from hyperlipidemia who would need hypolipemic treatment in case of preventive treatment and non treatment with simvastatin. Preventive treatment of hyperlipidemia with statins is most cost-effective and useful when compared to other methods of coronary heart disease treatment.

3

ŠTUDIJ ANALIZNEGA POSTOPKA ZA DOLOČEVANJE 1-OLEOIL-2-HIDROKSI-SN-GLICEROL-3-FOSFATA Z LC IN MS/MS TEHNIKO

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Študiral sem postopek za določevanje 1-oleoil-2-hidroksi-sn-glicerol-3-fosfata (LPA) s tekočinsko kromatografijo (LC) in večstopenjsko masno spektrometrijo (MS/MS). Diplomska naloga predstavlja izhodišče za razvoj metode za analizo LPA v krvnih vzorcih, ki bi pripomogla k zgodnjemu odkrivanju raka na jajčnikih.
Določil sem pogoje za detekcijo LPA standarda na kromatografski koloni na LC z detektorjem z nizom diod (DAD) ter z fluoroscenčnim detektorjem (FLD). Uporabljeni standard je imel koncentracijo 50 mg/l. Po literaturnih navedbah je zgornja koncentracija LPA bolnic z rakom na jajčnikih 19.8 mg/l. Določil sem pogoje za detekcijo standarda na MS/MS sistemu. Odzivnost signala je bila zadovoljiva, glavni fragmenti so imeli molsko maso: 79, 171, 153, 453. Detekcija na MS/MS sistemu je dala rezultate samo z elektrosprej negativno ionizacijsko nastavitvijo.
Analiza krvnih vzorcev LPA bi bila s to metodo možna. Za nadaljnje analize bi bilo primerno delati s t.i. Multi Reaction Monitoring načinom, kar pomeni, da bi pri analizi vzorca spremljali samo 153 m/z. Ta način bi bil zelo selektiven glede na zahtevno sestavo plazme. Za kvantitativno analizo je potrebno delati z metodo internega standarda.

STUDY OF ANALYTICAL PROCEDURE FOR 1-OLEOYL--2-HYDROXY- SN-GLYCEROL-3- PHOSPHATE DETERMINATION WITH LC AND MS/MS TECHNIQUE

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The analytical procedure for determination of 1-oleoyl-2-hydroxy-sn-glycerol-3-phosphate (LPA) using liquid chromatography (LC) and mass spectrometry (MS/MS) has been evaluated.
This work presents the starting point for LPA determination in blood samples, which should help in early ovarian cancer detection.
Analytical conditions for LPA detection on the LC column with diode array detector (DAD) and fluorescence detector (FLD) has been designated. 50 mg/l LPA was used as a standard. According to literature the highest LPA concentration of ovarian cancer patients is 19.8 mg/l. The analytical conditions for LPA detection on MS/MS have also been determined. Signal responses were satisfying, the main fragments have molecular masses of 79, 171, 153, 453, respectively. The detection of LPA with MS/MS was possible only, when the electrospray negative ionisation was used.
Analysis of LPA in blood samples would be possible using this method. For the LPA analysis of blood samples it would be appropriate to work with Multi Reaction Monitoring, which would mean that we monitor only 153 m/z. This method would be very selective considering the complex blood matrix. For quantitative analysis, it is necessary to work with the method of the internal standard.

3

DESIGNING THE STRAINS OF SUPERPRODUCING *E. COLI* NUCLEOSIDE PHOSPHORYLASES AND ELABORATION OF THE METHODS FOR THEIR PURIFICATION

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Drugs based on modified nucleosides are used for treatment of various cancer and viral diseases. Chemical synthesis of those substances is a difficult process requiring many stages. Glycosylation, resulting in binding of a heterocyclic base to an activated carbohydrate residue, is the key stage in the synthesis of nucleoside analogs. This stage can be performed using specific enzymes – nucleoside phosphorylases.
The aim of this work was to generate highly effective *E. coli* strains producing purine nucleoside phosphorylase, thymidine phosphorylase, uridine phosphorylase and to elaborate methods for the purification of these enzymes. To obtain the producing strain we used the Novagen's expression system. This system consists of plasmid pET in which target gene is under control of strong T7 promoter and the *E. coli* BL21(DE3) host strain is producing T7-RNA polymerase.
In the course of our research we have developed conditions which seem to be optimal for the biosynthesis of recombinant enzymes existing as soluble proteins retaining their native enzymatic activity. The enzyme preparations were purified by three steps including fractional precipitation with ammonium sulfate, followed by chromatography on Sephadex G-100 and DEAE-Sephacel.
Those enzymes have been used for the production of ribavirin.

3

RAZGRADNJA SLIK NA OSNOVI BARVNE INFORMACIJE

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Magistrska naloga je s področja računalniškega vida, t.j. obdelave in analize slikovnih vzorcev s pomočjo računalnika. Značilna aplikacija računalniškega vida v farmaciji je avtomatska vizualna kontrola kvalitete pakiranja tablet v pretisne omote, kjer je potrebno preveriti prisotnost tablet v pretisnih omotih ter določiti barvo, obliko, velikost in poškodovanost tablet. Ključni postopek obdelave, ki pomembno vpliva na kasnejše izločanje, analizo in interpretacijo slikovne vsebine, je segmentacija oziroma razgradnja slike. V nalogi so predstavljene različne barvne značilnice, ki opisujejo barvno informacijo ter postopki za razgradnjo barvnih slik v prostorih, ki jih značilnice določajo. Ker spektralne nehomogenosti osvetlitve prizora škodljivo vplivajo na natančnost, robustnost in hitrost razgradnje, naloga podrobno obravnava ta često prisotni pojav. Predstavljen je izvirni iterativni postopek razgradnje barvnih slik, ki temelji na neparametrični analizi prostora barvnih značilnic in retrospektivnih popravkih spektralnih nehomogenosti. V fazi učenja se poleg neparametričnega modela barvne informacije sistem samodejno nauči tudi parametrični model spektralnih nehomogenosti. S tem pridobimo dodatno znanje o prizoru, ki ga v postopku analize testnih slik pod enakimi pogoji osvetlitve uporabimo za hitro in natančno razgradnjo. Eksperimenti, ki so bili izvedeni na umetnih slikah, biomedicinskih slikah in slikah tablet v pretisnih omotih, so pokazali, da je predlagani postopek natančen, zanesljiv, robusten in hiter. Zaradi teh lastnosti je primeren tudi za uporabo v sistemih z računalniškim vidom, ki so namenjeni za avtomatsko vizualno kontrolo kvalitete izdelkov v zahtevni farmacevtski industriji.

IMAGE SEGMENTATION BASED ON COLOR INFORMATION

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The master thesis is from the field of computer vision, i.e. the area, which deals with image processing and analysis. Typical computer vision application in pharmaceutical industry is automated visual inspection of packing tablets into blisters. Features, like missing and broken tablets, tablet fragments, as well as the color, size, and shape of individual tablets have to be defined. Image segmentation, which is one of the basic image processing tasks, is very important for further extraction, analysis and interpretation of the image contents. Different color information features and algorithms for color image segmentation in feature spaces defined by these features are presented. The thesis also addresses the problem of the adverse effect of multispectral inhomogeneities (shading), reflecting themselves as smooth color variations, not present in original scene. Shading may influence the accuracy, robustness and speed of segmentation. A novel iterative color image segmentation method, based on nonparametric analysis of color feature space and retrospective correction of spectral shading is presented. In the training mode, the parametric model of the spectral inhomogenities and the nonparametric color model are automatically extracted. In this way additional information on the training image scene is obtained, which is later used to on-line accurately and robustly segment an image under the same illumination conditions. A number of experiments have been performed on artificial images, biomedical images and images of tablets in blisters. The results indicate that the proposed shading correction and segmentation methods are accurate, reliable, robust, and fast. Consequently, they are worth integrating into machine vision systems for analyzing color scenes in the pharmaceutical industry.

3

KOMUNIKACIJSKI VIDIKI EMBALAŽE. CELOSTNA GRAFIČNA PODOBA IZDELKOV ZA SONČENJE SUN MIX

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V vse večjem konkurenčnem boju se podjetja poslužujejo strategij, kako potrošniku uspešno prodati izdelek. Velik izbor istovrstnih izdelkov narekuje podjetjem, da z oblikovanjem embalaže prepričajo potrošnika. Teoretični del diplomske naloge opisuje načine, kako priti do dobre embalaže in prebuditi pri kupcu potrebo po nakupu.
Lahko bi se reklo, da embalaža odseva celoten splet marketinških aktivnosti podjetja in dostikrat je tudi edina, ki jo potencialni kupec zazna. Kupec velikokrat enači vsebino proizvoda z embalažo, zato mora biti le-ta oblikovana tako, da bo pritegnila njegovo pozornost, hkrati pa zadovoljila njegovo potrebo po kvaliteti, kakovosti, zaupanju, ... Embalaža je danes z ene strani propagandno sporočilo, z druge pa "prodajalec".
V nalogi je predstavljena raziskava konkurenčnih izdelkov za sončenje, ki so trenutno prisotni na slovenskem trgu. Vse izdelke sem razvrstila v tri cenovne razrede (višji, srednji in nižji). Sun Mix, linija za sončenje farmacevtskega podjetja Krka, spada v srednji cenovni razred. S preoblikovanjem njegove embalaže se poskušam približati višjemu cenovnemu razredu. Plastični embalaži sem dodala kartonsko embalažo, ki je značilnost višjega cenovnega razreda. Celostno grafično podobo linije za sončenje Sun Mix sem dopolnila s propagandnim in promocijskim materialom (mapa, prospekti, jumbo plakat, majica, dežnik, brisača).

COMMUNICATIVE ASPECTS OF PACKAGING. AN OVERALL GRAPHIC DESIGN OF SUN MIX SUN TAN PRODUCTS

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The fierce competition among different business concerns leads to the use of several selling strategies in order to persuade the customer to buy a particular product. The theoretical part of this diploma paper deals with the question of providing efficient packaging and the ability of arising the customer's need for the purchase.
One could claim that the packaging reflects all of the company's marketing acivities and that it is often the only stimulus to a customer's interest. The customer frequently equals the content and the packaging, thus it is of utmost importance that the packaging attracts the customer's attention and at the same time satisfies his need for quality and reliability. The packaging presents an advertisement and sells the product at the same time.
The practical part of my diploma work consists of a description and a research of several different sun-tan products available on Slovene market at present. The products have been classified into three different price categories (higher, middle, lower). Sun Mix, a sun-tan product by Krka can be classified into the middle price category. Remodelling of the packaging should place this product among high price products. A cardboard packaging typical for the higher price category has been added. The overall graphic design of Sun Mix sun-tan products was completed by other promotional items (a folder, a brochure, ...).

3

INFLUENCING SYSTEMIC AND PULMONARY HAEMODYNAMICS BY SHORT ISOMETRIC CONTRACTION AND VALSALVA MANEUVER IN PATIENTS WITH CHRONIC HEART FAILURE

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Aim: To assess the effect of short intensive isometric contraction on systemic and pulmonary haemodynamics in patients with chronic heart failure.
Set: 10 patients with severe form of chronic heart failure.
Methods: During a planned right side heart catheterization, the patients examined have undergone the following types of load: 2x maximum voluntary contraction of lower extremities muscles for 10 seconds with physiologic breathing, 2x solely Valsalva maneuver without the contraction of lower extremities muscles for 10 seconds, 2x the combination of maximum voluntary contraction of lower extremities muscles and Valsalva maneuver for 10 seconds. During each experiment, continuous synchronized record was taken of systemic blood pressure, pressure in pulmonary artery, ECG, breathing frequency and depth, change in volume of femoral and gastrocnemius muscles.
Results: Statistically, significant differences ($p<0.05$-ANOVA) were determined in all the four haemodynamic parameters evaluated (systemic systolic blood pressure, systemic diastolic blood pressure, systolic pressure in pulmonary artery, diastolic pressure in pulmonary artery) when comparing resting values versus Valsalva maneuver and when comparing resting values versus the combination of maximum voluntary contraction of lower extremities muscles + Valsalva maneuver. No significant differences ($p>0.05$-ANOVA) were determined when comparing resting values versus maximum voluntary contraction of lower extremities muscles and when comparing Valsalva maneuver versus the combination of maximum voluntary contraction of lower extremities muscles + Valsalva maneuver.
Conclusion: Short maximum voluntary contraction of lower extremities muscles in patients with severe chronic heart failure did not have any negative effect on systemic or pulmonary haemodynamics as far as physiologic breathing was maintained. Negative effects were caused by Valsalva maneuver.

3

KLONIRANJE IN IZOLACIJA REKOMBINANTNIH GIRAZ ZA RAZVOJ NOVIH GIRAZNIH INHIBITORJEV

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Sodoben pristop načrtovanja novih učinkovin temelji na poznavanju strukture in funkcije ključnih bakterijskih proteinov, med katere sodijo tudi DNA topoizomeraze II giraze. Glede na velik pomen giraz za preživetje mikroorganizma predstavljajo ti encimi perspektivno tarčo za oblikovanje novih protimikrobnih učinkovin. Za naše raziskave smo izbrali girazo *E. coli*, ki je sestavljena iz dveh proteinov, A in B, z molekulskima masama 97 in 90 kDa.
V prvem sklopu magistrske naloge smo uspešno pripravili nekaj miligramov visoko očiščenih in aktivnih rekombinantnih podenot giraz *E. coli* ter vzpostavili *in vitro* encimski testni sistem za merjenje učinkovitosti giraznih inhibitorjev. Pri tem se je modificirana metoda afinitetne kromatografije s kovalentno vezanim inhibitorjem, s pomočjo katere smo v eni stopnji očistili oba rekombinantna proteina, izkazala kot bolj enostavna in bolj kvalitetno, v primerjavi z metodo, ki jo navajajo v literaturi. Na osnovi rezultatov lahko potrdimo tudi prednost *in vitro* encimske metode v primerjavi z mikrobiološkim testiranjem in kot njihovo dopolnitev. Uporaba obeh metod nam bo omogočala sklepati na povezavo med strukturo in aktivnostjo substance ter istočasno analizirati bioaktivnosti novih giraznih inhibitorjev. V drugem sklopu magistrske naloge smo pripravili ekspresijski sistem za produkcijo N-terminalnega dela podenote B encima giraze *E. coli* z molekulsko maso 24 kDa (G24), ki lahko veže določen tip inhibitorjev. Z modificirano metodo afinitetne kromatografije smo v eni stopnji uspešno očistili preko 20 mg aktivnega rekombinantnega proteina na liter fermentacijske brozge. Strukturne študije z uporabo metod JMR so pokazale, da smo uspešno pripravili tudi fragment G24 označen z izotopom dušika (^{15}N), katerega uporaba kot material za določanje strukture kompleksov s potencialnimi inhibitorji omogoča načrtovanje in testiranje novih substanc na ravni molekularnih interakcij.

CLONING AND ISOLATION OF RECOMBINANT GYRASES FOR DEVELOPMENT OF NEW INHIBITORS

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Modern development of antimicrobial drugs relies on rational design of substances directed against specific targets such as topoisomerase II gyrases. These essential enzymes are present only in prokaryotes which make them a suitable target for antibacterial drugs. *E. coli* DNA gyrase, the focus of our work, is composed of two subunits, GyrA (97 kDa) and GyrB (90 kDa).
In the first part of our work we successfully prepared several milligrams of highly purified and active recombinant gyrase A and B subunits. We also prepared *in vitro* test system for the evaluation of activity of gyrase and its inhibitors. We showed that a modified affinity chromatography procedure based on an immobilized inhibitor was both more simple and efficient in comparison with purification procedure known from literature. Our results also confirm the advantage of *in vitro* enzyme test system in comparison to tests on microorganisms and particularly as its supplement. Based on these methods we are able to predict the structure-activity relationships of tested inhibitors and, at the same time, we are able to analyze its activity.
In the second part of our work we expressed the recombinant 24 kDa N-terminal domain of the B subunit of the DNA gyrase *E. coli* (G24), which had the ability to bind some inhibitors. Over 20 mg of highly purified and active recombinant protein were isolated from 1 L of *E.coli* culture by affinity chromatography with immobilized inhibitor. Structural data obtained by NMR confirmed that we have successfully prepared recombinant protein G24 labeled with ^{15}N. Structural studies of fragment-inhibitor complexes make possible to design novel inhibitors of DNA gyrase on the basis of defined molecular interactions.

3

PRIPRAVA KALUSNIH IN SUSPENZIJSKIH KULTUR RASTLINE *SOLANUM LACINIATUM* ZA BIOTEHNOLOŠKO PRIDOBIVANJE STEROIDNIH ALKALOIDOV

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Rastlinska vrsta *Solanum laciniatum*, ki spada v družino razhudnikovk *(Solanaceae)*, vsebuje veliko steroidnega alkaloida solasodina, ki se uporablja kot izhodna spojina za pridobivanje steroidnih učinkovin. Solasodin se v rastlini nahaja v obliki glikozida solasonina.
Pripravili in spremljali smo razvoj kalusne in suspenzijske kulture rastline *S. laciniatum*. Preizkusili smo osem različnih rastnih gojišč, glede na vsebnost rastlinskih rastnih regulatorjev. Rezultati so pokazali, da je najprimernejše gojišče za indukcijo in proliferacijo kalusne kulture MS osnovno gojišče s 3% saharozo, 1 µM KIN in 10 µM 2,4-D. Ugotovili smo, da ni razlike v rasti med suspenzijskimi kulturami, ki izvirajo iz različnih eksplantatov (list, steblo, korenina). Proučevali smo tudi vpliv različnih koncentracij saharoze in ugotovili, da saharoza vpliva na rast suspenzijske kulture.
Kot analizno metodo za določanje vsebnosti steroidnih alkaloidov smo uporabili kapilarno elektroforezo. Ugotovili smo, da je v kulturi nastalo več različnih spojin, ki so po elektroforetskih lastnostih podobne solasoninu. Morda so to različni glikozidi solasodina. Z elicitacijo (salicilna kislina, jasmonska kislina) nismo dosegli povečanja vsebnosti solasonina v suspenzijski kulturi. Pri visokih koncentracijah jasmonske kisline se je vsebnost celo močno zmanjšala. Zato so na tej stopnji potrebne še nadaljnje raziskave v smeri odkritja eksogenega induktorja (elicitorja) steroidnih alkaloidov v vrsti *Solanum laciniatum*.

DEVELOPMENT OF CALLUS AND SUSPENSION CULTURES OF *SOLANUM LACINIATUM* FOR BIOTECHNOLOGICAL PRODUCTION OF STEROIDAL ALKALOIDS

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Plant species *Solanum laciniatum*, the member of the *Solanaceae* family, contains a steroidal alkaloid solasodine, which is used as raw material for the production of steroidal medicines. In the plant, solasodine is in the form of glycoside solasonine.
Within the scope of this study, a development of callus and suspension cultures of *Solanum laciniatum* was prepared and monitored. Eight different growth media, supplemented with various plant growth regulators, were tested. Results indicates that the most suitable medium for induction and proliferation of callus is MS medium, supplemented with 3% sucrose, 1 µM KIN and 10 µM 2,4-D. We found out that there is no significant difference in growth among suspension cultures, which initiate from different explants (leaf, stem, root). But we did find out the influence of different sucrose concentration on the growth of plant cells in suspension culture.
Capillary electrophoresis (CE) method was used for the determination of steroidal alkaloids. Several substances, related to solasonine (in respect of CE mobility) were detected in the culture, which might represent different solasodine glycosides. By means of elicitor addition (salicylic acid, jasmonic acid), the expected induction of solasonine biosynthesis was not achieved. Even more, by using a higher concentration of jasmonic acid we observed a decrease of solasonine content in suspension culture. Further improvement need to be performed by trying to discover novel inductors and/or elicitors of steroidal alkaloids in *Solanum laciniatum*.

3

UVEDBA IZLUŽEVALNEGA TESTA PRI OCENI KVALITETE ODPADNEGA BLATA IZ INDUSTRIJSKE BIOLOŠKE ČISTILNE NAPRAVE

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Vse hitrejši razvoj tehnologij čiščenja odpadnih vod narekuje tudi ustrezen način ravnanja z odpadnim blatom iz čistilnih naprav. Po predpisih EU obstajajo določene omejitve glede odlaganja blata na deponijah, predvsem pa glede uporabe na kmetijskih površinah. Te omejitve se nanašajo predvsem na vsebnost težkih kovin, ki se v blatu akumulirajo.
Namen mojega raziskovalnega dela je bila ocenitev kvalitete odpadnega blata glede na našo zakonodajo in vpeljava izluževalnega testa, ki predstavlja začetno fazo v pripravi vzorcev, v katerih sem določil vsebnost težkih kovin. Vsebnost težkih kovin v blatu in izlužku je bila določena z AAS tehniko (atomska absorpcijska spektrometrija), ki temelji na pojavu, da prosti atomi absorbirajo svetlobo določene valovne dolžine, ki je karakteristična za posamezen element, v mojem primeru kovino.
Na osnovi dobljenih rezultatov in glede na veljavno zakonodajo ugotavljam, da sodi blato iz čistilne naprave Krka, d. d., Novo mesto v skupino nenevarnih odpadkov in je primerno za odlaganje na odlagališču za nenevarne odpadke.

INTRODUCTION OF THE LEACHING TEST TO ESTIMATE THE QUALITY OF WASTE SLUDGE FROM INDUSTRIAL BIOLOGICAL WASTE WATER TREATMENT

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The rapid development of waste water treatment technology dictates the need for the management of waste sludge in an economically and environmentally acceptable manner. According to EU directives there exist some limitations on available practices for the management of sewage sludge, like landfilling and especially agricultural use. Heavy metals which could be accumulated in the sludge are of the greatest concern.
The purpose of the research was to evaluate the quality of sludge from an industrial biological waste water treatment plant according to our legislation and also to put the leaching test into the laboratory practice. The leaching test was the first step for sample preparation and important for heavy metals detection. The amount of heavy metals was detected by AAS technique. This technique is based on facts that atoms absorb the light of specific wavelenght, which is specific for each element in my case heavy metal.
According to results of this study and to Slovenian legislation the sludge from waste water treatment plant in Krka can be ranged among nonhazardous wastes and is therefore suitable for disposal on sanitary landfill.

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OPTIMIZACIJA REOLOŠKIH LASTNOSTI HIDROFILNIH IN LIPOFILNIH MIKROEMULZIJ ZA DERMALNO UPORABO

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Mikroemulzije predstavljajo sodobne nosilne sisteme z veliko prednostmi. So temodinamsko stabilne, bistre, enostavno jih je pripraviti in imajo veliko sposobnost solubilizacije učinkovin. Pri dermalni aplikaciji vplivajo na prehod učinkovine v kožo, vendar so pogosto preveč tekoče za enostaven nanos, zato je potrebno njihove reološke lastnosti optimirati.
Namen raziskovalne naloge je bil poiskati najprimernejše zgoščevalce, ki bi povečali viskoznost mikroemulzij, obenem pa kar najmanj vplivali na njihovo stabilnost in ostale fizikalno-kemijske lastnosti. Modelni mikroemulziji sta bili sestavljeni iz istih komponent (Miglyol 812® kot lipofilna in bidestilirana voda kot hidrofilna faza, emulgatorski film pa sta sestavljala Labrasol® in Plurol oleique® v zmesi 4:1), vendar v različnih razmerjih, tako da smo izdelali V/O in O/V sistem. Izmed preizkušenih zgoščevalcev za hidrofilne mikroemulzije se je kot najprimernejši izkazal ksantan v 0,5% m/m koncentraciji, za lipofilne pa Aerosil® v 4% m/m koncentraciji. Mikroemulzije brez zgoščevalcev izkazujejo lastnosti idealnih, Newtonskih tekočin. Mikroemulzije zgoščene z izbranimi zgoščevalci v navedenih koncentracijah, pa so izkazovale plastičnen pretok, ki je značilen za prisotnost šibke gelske strukture. Ugotovljeno tokovno obnašanje so potrdili tudi mehanski spektri pridobljeni z metodo dinamične oscilacijske reometrije. Sproščanje modelne učinkovine natrijevega askorbilfosfata smo spremljali v modelu *in vitro*. Količina učinkovine, sproščene iz hidrofilne mikroemulzije se je ob dodatku zgoščevalca zmanjšala, pri lipofilni mikroemulziji pa povečala, kar je posledica spremembe prevodnosti zunanje, oljne faze. Slednje smo potrdili tudi z merjenjem električne prevodnosti. Kinetika sproščanja je v vseh primerih v skladu s √t redom (Higuchijeva kinetika).
Kot ustrezen zgoščevalec za lipofilne mikroemulzije se je izkazal Aerosil®, medtem ko ksantan v primeru hidrofilnih ni izpolnil vseh zahtev, zato bo na tem področju potrebno delo nadaljevati.

OPTIMIZATION OF RHEOLOGICAL PROPERTIES OF HYDROPHILIC AND LIPOPHILIC MICROEMULSIONS FOR DERMAL USE

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Microemulsions as drug-delivery systems have a lot of advantages: they are thermodynamically stable, easy to prepare and they improve drug solubility. If applied topically, they can influence the penetration of the drug in the skin. Their dermal application, however, is somehow difficult because of their low viscosity. Therefore it is advisable to optimize their rheological properties.
We tested some potential thickeners for hydrophilic and lipophilic microemulsions composed of Miglyol 812® as oil phase, bidistilled water as aqueous phase, Labrasol® as emulsifier and Plurol oleique® as co-emulsifier. The most appropriate thickener for lipophilic microemulsion was Aerosil® in 4% concentration and xantan in 0,5% concentration for hydrophilic microemulsions. Microemulsions without thickeners exhibited ideal behavior whilst microemulsions with added thickener in stated concentrations exhibited plastic behavior, typical for weak gel structures. The release of model drug, sodium ascorbilphosphate, from the thickened microemulsions increased form 30% to 54% for lipophilic microemulsions and decreased from 81% to 62% for hydrophilic microemulsions. Release kinetics corresponded to √t order (Higuchi kinetics) in all cases, but modified microemulsions were more in accordance with the model.
Aerosil® proved be a suitable thickener for lipophilic microemulsions, but xantan as a thickener for hydrophilic microemulsions did not fulfill all the requirements, therefore further studies are required.

3

EKONOMSKI UČINKI POENOSTAVLJENIH CARINSKIH POSTOPKOV V PODJETJU KRKA

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Poenostavitve carinskih postopkov pomenijo za podjetje racionalno poslovanje, bistveno zmanjšujejo stroške, posledično povečujejo konkurenčnost podjetja ter pomenijo prilagoditev podjetja novim razmeram v Evropski uniji (EU). Carina je bila dolžna sprejeti novosti z ozirom na vstop v EU, saj je carinski sistem eno ključnih področij pri prilagajanju za polnopravno članstvo Slovenije v EU.
Prilagajanju slovenske carine EU so primorana slediti tudi podjetja. Uvedba poenostavljenih carinskih postopkov je prinesla naslednje prednosti podjetju in carini: zmanjšanje stroškov pri poslovanju, hitrejše izvajanje postopkov, manjši obseg papirne dokumentacije, večja preglednost in kvaliteta podatkov in "čimbolj neopazen" carinski postopek pri mednarodnem pretoku blaga.
Stroškovno sem ovrednotila obe vrsti izvajanja postopkov, tako običajnega in poenostavljenega carinskega postopka pri uvozu in izvozu ter naredila primerjavo.
Finančna primerjava je pokazala veliko znižanje stroškov v prid poenostavljenih postopkov pri uvozu in izvozu. Prihranek je odvisen od števila uvoznih in izvoznih pošiljk. Izvajanje poenostavitev je časovno hitreje, blago je hitreje ocarinjeno ter podjetju omogoča direktno dobavo pošiljk v tujino in iz tujine.
Optimalna poenostavitev na obravnavanem področju pa je brezpapirno poslovanje med podjetjem in carino.

ECONOMIC EFFECTS OF SIMPLIFIED CUSTOMS PROCEDURES IN THE ENTERPRISE KRKA

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Simplifications of customs procedures mean for the enterprise rational business operations, they decrease essentially the costs, consequently they increase competitiveness of the enterprise and mean the adaptation of the enterprise to new relations in the European Union (EU). The customs were obliged to accept the novelties with respect to the entry in the EU as the customs system is one of the key fields in the adaptation for full membership of Slovenia in the EU.
The enterprises are forced to follow the adaptation of Slovene customs to the EU. The introduction of simpliefied customs procedures brought the following advantages both to the enterprise and the customs: cost decrerase in business operations, quicker performance of procedures, less paper documentation, greater surveyability and quality of data and "as imperceptible" customs procedure as possible in international flow of goods.
As regards the costs I evaluated both the usual and the simplified performance of customs procedure in the import and export of goods and compared them.
The financial comparison showed a great reduction of costs on behalf of simplified procedures both in import and export. The saving depends on the number of imported and exported consignments. The performance of simplified procedures takes less time, the assessment of duty payable on goods is quicker and enables the enterprise direct importing and exporting.
The optimal simplification in the field of customs is paperless business operation between the enterprise and customs.

3

ŠTUDIJ OKSIDACIJE IN STABILIZACIJE OKSIDATIVNO NESTABILNEGA HIPOLIPEMIKA Z ANTIOKSIDANTI NARAVNEGA IZVORA

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Proučevali smo oksidacijsko občutljivost lovastatina, zaviralca HMG-CoA reduktaze, v trdnem stanju. Z variranjem časa inkubacije smo proučevali časovni potek zmanjševanja vsebnosti neoksidiranega lovastatina v oksidativni atmosferi in ugotovili, da je lovastatin pri povišani temperaturi oksidativno nestabilna zdravilna učinkovina. Prav tako smo raziskali vpliv temperature na hitrost oksidacije. Dokazali smo, da lahko oksidacijo lovastatina v trdnem agregatnem stanju učinkovito zavremo z uporabo antioksidantov rastlinskega izvora, med katerimi smo izbrali rutin, kvercetin, galno, kavno in askorbinsko kislino. Preskušana antioksidanta flavonoidnega tipa smo v študijo vključili zaradi njihovega dobro znanega antiinflamatornega, antivirusnega, antibakterijskega, protikancerogenega, antioksidativnega in antiradikalskega delovanja in pozitivnega vpliva na inhibicijo oksidacije LDL, ki je ključnega pomena za razvoj in napredovanje ateroskleroze ter tako sinergistično deluje z lovastatinom. Ugotavljali smo učinkovitost stabilizacije posameznega preskušanega antioksidanta v odvisnosti od količine, razliko med učinkovitostjo posameznih antioksidantov v zmesi ter tableti in pri tem uporabili različna analitska orodja. Kot najučinkovitejši antioksidant v trdnem stanju se je v vseh preskušenih količinskih razmerjih izkazala kavna kislina, ki ji sledijo rutin, galna kislina, kvercetin ter askorbinska kislina z majhnim antioksidativnim učinkom. Razvili smo kromatografsko metodo za določevanje vsebnosti lovastatina in jo uporabili tudi za identifikacijo glavnih oksidacijskih produktov s pomočjo LC/MS/MS tehnike ter alternativno metodo z uporabo kapilarne elektroforeze in primernost te metode potrdili z njeno validacijo. Primerjali smo rezultate obeh analitskih metod ter ocenili njuno natančnost, točnost ter primernost v obravnavanem primeru.

STUDY OF OXIDATION AND STABILIZATION OF OXIDATIVE UNSTABLE HYPOLIPEMIC DRUG WITH NATURAL ANTIOXIDANTS

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Oxidative stability of HMG-CoA reductase inhibitor lovastatin in the solid state has been examined. Changing the time and temperature of oxidation and following the decomposition of our model drug lovastatin we have found out that lovastatin is unstable to oxidation under higher temperatures and in the presence of oxygen and that some antioxidants, such as rutin, quercetin, gallic, caffeic and ascorbic acid markedly stabilize the drug. Tested flavonoid antioxidants have been involved in the study because of their known and interesting medicinal properties such as antiinflammatory, antiviral, antibacterial, antitumor activities, antioxidative, antiradical and important positive effect on LDL oxidation and this having synergistic effect on lovastatin. Using different comparative analitical tools, the oxidative effect of tested antioxidant in the binary mixuters and in tablets was examined. The most significant antioxidative effect was obtained with caffeic acid and rutin, followed by gallic acid and quercetin, although the antioxidant activity of all four antioxidants tested, except ascorbic acid with only moderate effect, is relatively high. Identification of the main oxidation products of lovastatin by LC/MS/MS and the development of comparative capillary electrophoretic method, its validation and its quality and quantitative comparison to HPLC method has been performed.

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SINTEZA NOVOBIOCINU SORODNIH SPOJIN

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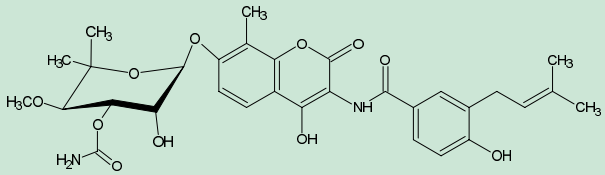
Novobiocin (**1**) je kumarinski antibiotik, katerega zaradi njegove toksičnosti in pojave rezistence že dolgo ne uporabljajo v klinični praksi. Zaradi njegove aktivnosti proti MRSA pa so njemu sorodne spojine tarča številnih raziskav. V našem delu smo študirali številne aspekte sinteze novobiocinu sorodnih spojin.
Iskali smo najprimernejšo metodo za tvorbo glikozidne vezi v tovrstnih spojinah. Pri tem smo ugotovili, da je trikloroacetimidatna metoda zelo primerna za naš namen, saj smo z njo uspešno pripravili modelne spojine 4-deoksinovobiocinskega tipa.[1]
Razvijali smo lastno metodo za sintezo novioze, katere derivat je sladkorna komponenta novobiocina.
Raziskovali smo novo sintezo derivata 3-amino-4-hidroksikumarina, ki služi kot osnova za pripravo drugih kumarinskih komponent.
Nekatere kumarine smo derivatizirali pod vplivom mikrovalov.[2]



SYNTHESIS OF NOVOBIOCIN-RELATED COMPOUNDS

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Novobiocin (**1**) is a coumarin-containing antibiotic, which is due to its toxicity and the development of resistance not anymore employed in clinical praxis. Recently, because of its anti-MRSA activity some further studies of novobiocin-related derivatives were launched. We studied various aspects of the synthesis of novobiocin-related compounds.
We were searching for the most convenient method for the formation of the glycosidic linkage in such compounds. We found the trichloroacetimidate methodology to be very useful for our purpose; it was successfully applied for the synthesis of some model 4-deoxynovobiocine derivatives.[1]
We have developed a new synthetic methodology for the preparation of noviose, the sugar moiety of novobiocin.
We investigated a new synthesis of a derivative of 3-amino-4-hydroxycoumarin, which could be used for the preparation of some other coumarin moieties.
Some coumarins were functionalysed under microwave conditions.[2]

[1]M. Ješelnik, J. Plavec, S. Polanc, M. Kočevar, *Carbohydr. Res.*, **2000**, 328, 591.
[2]M. Ješelnik, R. S. Varma, S. Polanc, M. Kočevar, *Chem. Commun.*, v tisku.

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TELESNA SESTAVA PRAŠIČEV KRŠKOPOLJSKE PASME

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Avtohtone pasme domačih živali so del biotske raznovrsti ter naša naravna in kulturna dediščina. Pri vrsti prašič je krškopoljski prašič edina slovenska avtohtona pasma. Nastala je na področju Dolenjske, kjer je v omejenem obsegu tudi ohranjena. V zadnjih desetih letih je pasma krškopoljski prašič zaradi ohranjanja biotske raznovrstnosti domačih živali pridobila na pomenu in biološke značilnosti pasme so predmet številnih raziskav. Del teh proučevanj je vključenih tudi v mojo diplomsko nalogo.
V posebnem poskusu s krmljenjem od libitum je bila na vzorcu 10 prašičev krškopoljske pasme raziskana splošna zmogljivost rasti do večjih telesnih mas, potek rasti v času pitanja, posebna zmogljivost rasti nekaterih telesnih tkiv (mišičja in maščobnega tkiva), telesne mere klavnih polovic in mere kakovosti mišičja (pH, barva, električna prevodnost in vodenost). Meritve smo primerjali z meritvami istih lastnosti na 10 prašičih, komercialnih križancih. Z genskim testom za sindrom maligne hipertermije smo ugotavljali frekvenco teh genov pri krškopoljskih prašičih v poskusu.
Prašiči krškopoljske pasme imajo veliko splošno zmogljivost rasti do večjih telesnih mas. Frekvenca genov za sindrom maligne hipertermije je bila pri krškopoljskih prašičih sorazmerno visoka (p = 30%). Ugotovili smo statistično značilen vpliv pasme, spola in mase klavnih polovic na maso in sestavo posameznih klavnih delov ter lastnosti mesa. Krškopoljski prašiči so imeli lažje stegno in težjo glavo, manj mišičnega tkiva ter več podkožnega in medmišičnega maščobnega tkiva kot komercialni križanci. Krškopoljski prašiči so imeli svetlejše meso in manjšo sposobnost dolge hrbtne mišice za vezavo vode.

BODY COMPOSITION OF KRŠKOPOLJE PIG

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The autochthonous breeds of domestic animals are part of biotic diversity and they are also our natural and cultural inheritance. The Krškopolje pig is the only autochthonous breed of pig species with its origin in Dolenjska where a limited number is preserved. In the past ten years the importance of the Krškopolje pig has grown because of the need to preserve the biotic diversity. The breed´s biotical characteristics are subject of scientific research. Part of these are included in my graduation thesis.
In a special feeding experiment (ad libitum) we researched the growth rate, body measures, growth characteristics of meat and fat tissue and measures of meat quality (pH, color, electric translation and water retaintion) on a sample of 10 Krškopolje pigs. We compared them to a group of another 10 pigs, commercial crossbreeds. We made a genetic test for Syndrome malign hypertermy (SMH) on the researched Krškopolje pigs.
The Krškopolje pig has a large general growing capacity to heigher weight and his frekvency of SMH gene was high (p = 30%). The breed, gender and carcass weight influence on the weight and tissue percentage of carcass cuts and meat characteristics were statistically determined. If compared to the commercial crossbreeds, the Krškopolje pig had a lighter leg, heavier head, less meat, more subcutaneous and intermuscular fat. The Krškopolje pig had brighter meat, with lower water retaintion.

3

ŠTUDIJ DIREKTNEGA PELETIRANJA V LABORATORIJSKEM HITREM MEŠALNIKU

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Pelete so zaradi številnih prednosti pred ostalimi farmacevtskimi oblikami postale pomemben del farmacevtskega razvojnega dela.
V okviru raziskovalne naloge smo izdelali pelete z direktnim peletiranjem v hitrem mešalniku. S pomočjo faktorske analize smo preučevali vplive izbranih faktorjev (tip veziva, količina dodanega veziva, čas gnetenja peletne zmesi in količina mikrokristalne celuloze (MCC) v peletni zmesi) na opazovane parametre pelet. Izračunali smo tudi glavne učinke faktorjev ter njihove interakcije. Z namenom upočasnitve hitrosti sproščanja teofilina smo pelete obložili z dvema različnima polimernima oblogama (Eudragit RS/RL in Surelease). V okviru naloge smo želeli preizkusiti tudi vpliv tipa polimera in debeline obloge na hitrost sproščanja.
Z analizo izdelanih pelet smo ugotovili, da se s spremembo tipa veziva (PVP-HPC) ter s podaljšanjem časa gnetenja poveča delež pelet v našem optimalnem področju. Prav tako smo ugotovili, da s povečanjem količine dodanega veziva ter količine MCC v peletni zmesi dobimo manjši delež pelet v želenem velikostnem območju. Hitrost sproščanja aktivne substance iz pelet je padala v sorazmerju s povečanjem debeline polimerne obloge. Z medsebojnim primerjanjem polimernih oblog smo ugotovili, da je Eudragit RS/RL kot polimerna obloga bolj primeren, saj se je iz pelet z Eudragitno oblogo v 12 urah sprostila večina teofilina.

STUDY OF DIRECT PELLETIZATION IN A LABORATORY HIGH-SHEAR MIXER

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As pellets have several advantages over other pharmaceutical formulation, they have become an important part of pharmaceutical development work.
In our study, pellets were obtained by direct pelletization in a high-shear mixer. The effects of selected factors (type of binder, quantity of added binder, time of kneading and quantity of microcrystalline cellulose (MCC) in the pellet mixture) on some physical parameters of the pellets were studied. In order to prolong the release of the active substance, the pellets were coated with two different polymer coatings (Eudragit RS/RL and Surelease). The effects of the type of binder and the thickness of polymer layer on dissolution rate were determined for the pellets.
The analysis of pellets indicated that a change of the type of binder (PVP-HPC) and increasing of the time of kneading resulted in an increased portion of pellets in our optimal area. If the quantity of added binder and the quantity of MCC in the pellet mixture were increased, the portion of pellets in our optimal area decreased. The dissolution rate decreased proportionally to increased thickness of the polymer layer. A comparison of the polymer layers showed that Eudragit RS/RL was more suitable for prolonging the release of the active substance from pellets.

3

UPORABA BOX-BEHNKEN STATISTIČNEGA MODELA ZA OPTIMIZACIJO VODNEGA OBLAGANJA PELET Z VGRAJENIM DIKLOFENAKOM

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Optimizacija farmacevtske oblike, ki temelji na empiriji, je dolgotrajen in zelo težaven proces. Pri našem delu smo se odločili za uporabo statističnega modela kot orodja, s pomočjo katerega bomo optimizirali vodno oblaganje diklofenak pelet s podaljšanim sproščanjem.
Za oblaganje peletnih jeder smo uporabljali laboratorijski granulator z vrtinčenjem. Eksperimente smo načrtovali z Box-Behnken tri-faktorskim načrtom na treh nivojih. Kot neodvisne spremenljivke smo izbrali koncentracijo trietil citrata v vlogi plastifikatorja, razmerje metakrilatnih polimerov (Eudragit RS : RL) ter količino nanešene disperzije v procesu oblaganja. Naše izbrane odvisne spremenljivke so bile kumulativne vrednosti za % sproščenega diklofenaka po 3, 4 in 6 urah. Tako smo za posamezni vzorec iz načrta eksperimentov dobili pripadajoč profil sproščanja. Z metodo odgovornih površin smo poiskali povezavo med odvisnimi in neodvisnimi spremenljivkami. Izbrani model je za naš iskani optimum t.j. 40% sproščene učinkovine po 3 urah sproščanja, izbral ustrezno sestavo filmske obloge, ki je predvidevala 25% trietil citrata, razmerje Eudragitov RS in RL 3/1 in količino uporabljene disperzije v procesu oblaganja 400 g. Pripravili smo nov vzorec s sestavo, ki jo je podal računalniški program ter izračunane vrednosti profilov sproščanja primerjali z eksperimentalnimi. Ugotovili smo, da je prekrivanje rezultatov dobro. Za dodatni študij izdelanih pelet in filmskih oblog smo uporabili elektronsko mikroskopijo, dinamično sorpcijo vode, merjenje stičnega kota in dinamično diferenčno kalorimetrijo z modulacijo temperature.
Ocenjujemo, da je uporaba Box-Behnken statističnega modela z metodo odgovornih površin pomemben in učinkovit pripomoček, ki s sistematičnim pristopom skrajša čas potreben za razvoj farmacevtske oblike in izboljša naše raziskovalno-razvojno delo.

OPTIMISATION OF AQUEOUS COATING OF DICLOFENAC PELLETS USING BOX-BEHNKEN STATISTICAL MODEL

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Optimisation of a pharmaceutical dosage form that is based on empirical work is a time consuming and very hard process. A statistical model was used in our study as a tool of optimisation of aqueous film coating of diclofenac pellets with prolonged release.
Film coating of pellet cores was performed in a laboratory fluid bed apparatus. The chosen independent variables, i.e. plasticizer (triethyl citrate) concentration, methacrylate polymers ratio (Eudragit RS:RL) and the quantity of coating dispersion, were optimised using a three-factor, three-level Box-Behnken design. The chosen dependent variables were cumulative percentage values of diclofenac dissolved in 3, 4 and 6 h. Based on the experimental design, different diclofenac release profiles were obtained. Response surface plots were used to relate the dependent and the independent variables. The optimisation procedure generated an optimum of 40% release in 3 h where the levels of plasticizer concentration, quantity of coating dispersion and polymer to polymer ratio (Eudragit RS:RL) were 25% w/w, 400g and 3/1, respectively. The optimised formulation prepared according to computer-determined levels provided release profiles that were close to the predicted values. The formulations and also film coatings were further studied by scannig electronic microscopy, dynamic water sorption measurments, contact angle measurments and modulated temperature differrentialscanning calorimetry.
It can be concluded that Box-Behnken statistical model with the analysis of response surfaces is a powerful, efficient and systematic tool that shortens the time required for the development of pharmaceutical dosage forms and improves research and development work.

3

PREVERJANJE VPLIVA SPREMENLJIVK NA ODSTRANJEVANJE DUŠIKOVIH SPOJIN V SISTEMIH S PRITRJENO BIOMASO

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Vpliv posameznih spremenljivk na odstranjevanje dušikovih spojin iz odpadnih vod je znan, toda pomembnost vpliva posameznih spremenljivk ni raziskana.
Preskusi so bili izvedeni s kombiniranim reaktorjem. Določitev soodvisnih spremenljivk je bila narejena s korelacijskimi metodami. Z neodvisnimi spremenljivkami so bili narejeni preskusi po Plackett-Burmanovem eksperimentalnem načrtu. Sledil je prikaz vpliva posameznih spremenljivk na hitrost nitrifikacije in denitrifikacije.
Linearno odvisni sta bili koncentracija raztopljenega kisika v aerobni coni in na vrhu reaktorja ter koncentracija amonijevega in Kjeldahlovega dušika. Na hitrost nitrifikacije je najbolj vplivala hitrost zraka, sledili sta hitrost tekočine in koncentracija kisika v aerobni coni. Koncentracija Kjeldahlovega dušika ni imela vpliva na hitrost nitrifikacije. Največji vpliv na hitrost denitrifikacije je imela hitrost tekočine, manjšega KPK in še manjši vpliv je imela koncentracija kisika v anoksični coni. Koncentracija $N\text{-}NO_x$ ni vplivala na potek denitrifikacije.
Poznavanje vpliva spremenljivk nam omogoča načrtovanje laboratorijskih preskusov, ocenjevanje uporabnosti modelov za dimenzioniranje čistilnih naprav s pritrjeno biomaso, upravljanje industrijskih čistilnih naprav in optimiziranje odstranjevanja dušikovih spojin iz odpadnih vod.

EVALUATION OF EFFECT OF VARIABLES ON NITROGEN REMOVAL BY BIOFILM PROCESS

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The influence of single variables on a nitrogen removal from wastewaters is well-known but the importance of the influence of single variables isn't researched.
The study was performed in a combined reactor. Definition of dependent variables was done with correlation methods. A Plackett and Burman design was used for the evaluation of the influence of independent variables on the nitrogen removal. A presentation of the influence of single variables on the nitrogen removal was done.
The concentration of dissolved oxygen in aerobic zone was linearly related with the concentration of dissolved oxygen at the top of the reactor and the concentration of ammonium nitrogen was linearly related with the concentration of Kjeldahl nitrogen. Air velocity had the main influence on the nitrification rate. Liquid velocity and concentration of dissolved oxygen in aerobic zone had less influence. The effect of the concentration of Kjeldahl nitrogen was unimportant. Liquid velocity had the main influence on the denitrification rate. COD and concentration of dissolved oxygen in anoxic zone had less influence. The effect of the concentration of $N\text{-}NO_x$ was insignificant.
Knowledge of the influence of single variables on the nitrogen removal is useful for planning of pilot tests, estimation of models, running of industrial wastewater treatment plants and process optimization.

3

SINTEZE APLISINOPSINOVIH ANALOGOV. PRETVORBE 5-[(DIMETILAMINO)METILIDEN]IMIDAZOLIDIN-2,4-DIONOV IN SORODNIH SPOJIN

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Aplisinopsini so naravne spojine, prvič izolirane iz morskih spužev *Aplysinopsis reticulata* in *Verongia spengelli*, ki kažejo citotoksičnost napram rakastim celicam in delujejo kot vzpodbujevalci nevrotransmiterjev. Tudi nekatere aplisinopsinu sorodne spojine so heterocikli z biološko pomembno aktivnostjo, saj se pojavljajo v osnovnih strukturah naravnih alkaloidov, npr. alkorneina, kot komponente v t-RNA in kot analogi aciklovira, ki prav tako kažejo antiherpetično aktivnost.
V okviru diplomskega dela sem s preprostim sinteznim postopkom, pri katerem izhajamo iz 5-[(dimetilamino)metiliden]hidantoinskih derivatov, pripravila derivate aplisinopsina, pri katerih je indolni obroč zamenjan z različnimi heterocikličnimi sistemi.
Pri reakciji hidantoina z bis(dimetilamino)-*t*-butoksimetanom (Bredereckovim reagentom) oz. DMFDMA sta nastala 5-[(dimetilamino)metiliden]imidazolidin-2,4-dion oz. 5-[(dimetilamino)metiliden]-3-metilimidazolidin-2,4-dion. Sledila je izmenjava dimetilaminske skupine z različnimi C-nukleofili: barbiturno kislino, 4-hidroksi-6-metilpiran-2-onom, 1-fenil-3-metilpirazolonom, 1,3-difenilpirazolonom, 2-piridilacetonitrilom, 2-hidroksi-1,4-naftokinonom in 4-hidroksipiridin-2-onom. Vse reakcije so potekle v ocetni kislini pri temperaturi vrelišča, ustrezni aplisinopsinovi analogi pa so nastali stereoselektivno v (Z) konfiguraciji. Dobljeni celokupni izkoristki so bili relativno visoki, od 43-91 %.

SYNTHESIS OF APLYSINOPSIN ANALOGS. REACTIONS OF 5-[(DIMETHYLAMINO)METHYLIDENE]-IMIDAZOLIDINE-2,4-DIONES AND RELATED COMPOUNDS

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Aplysinopsines, isolated from marine organisms, are interesting compounds due to their specific biological activity.
A simple two step method was developed for the preparation of aplysinopsin analogs. In the reaction with bis(dimethylamino)-*t*-butoxymethane or DMFDMA, hydantoin was converted to 5-[(dimethylamino)methyilidene]imidazolidine-2,4-dione and to 5-[(dimethylamino)methylidene]-3-methylimidazolidine-2,4-dione. The dimethylamino group in both compounds could easily be exchanged with an active methylene group of C-nucleophilic heterocyclic compounds: barbituric acid, 4-hydroxy-6-methylpyran-2-one, 3-methyl-1-phenylpyrazolone, 1,3-diphenylpyrazolone, 2-pyridilacetonitrile, 2-hydroxynaphthoquinone and 4-hydroxypyridin-2-one. The reactions were carried out in boiling acetic acid and new aplysinopsin analogs were obtained stereoselectively in (Z) form in 43 to 91 % yields.

3

POLITIKA OGLAŠEVANJA IZDELKOV ZA SAMOZDRAVLJENJE V FARMACEVTSKI INDUSTRIJI

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Zaradi vse večje konkurence na trgu zdravil za samozdravljenje ni več dovolj le kvaliteta izdelka, pač pa je pomembno tudi oglaševanje, ki v kupcu vzbudi zanimanje in občutek dobrega izdelka.
V okviru diplomskega dela sem analizirala drugačen položaj farmacevtske industrije pri oglaševanju zdravil za samozdravljenje, v primerjavi z oglaševanjem izdelkov katerekoli druge panoge. V praktičnem delu sem prikazala vlogo javnega oglaševanja t.i. preklopnih zdravil v podjetju Krka, d. d., vključno s predstavitvijo promocijske akcije blagovne znamke Panatus v Sloveniji in sicer t.i. "above the line" del promocije ter tako potrdila postavljeno trditev, da je povečanje tržnega deleža blagovne znamke nedvomno posledica učinkovite oglaševalske akcije.
Ugotovila sem, da se oglaševalska politika Krke, d. d. znotraj programa OTC razlikuje glede na končni cilj, ciljno skupino, konkurenco na trgu, okolje, v katerem delujemo itd. Je rezultat jasno postavljenih tržnih in komunikacijskih ciljev. V oglaševalski politiki se pojavljajo vsi štirje elementi trženjskega upravljanja in sicer analiza, načrtovanje, izvajanje in nadzor. V oglaševalski akciji blagovne znamke Panatus smo z ustrezno oglaševalsko politiko izkoristili trenutek prerazporeditve zdravil iz pozitivne na negativno listo in s politiko ciljnega oglaševanja povečali tržni delež izdelka.
Poleg uveljavljanja blagovne znamke proizvoda se z ustrezno oglaševalsko politiko utrjuje tudi dobro ime podjetja, kar vpliva na pospeševanje prodaje in s tem na boljši poslovni uspeh, kar je končni cilj oglaševanja.

ADVERTISING POLICY CONCERNING SELF-MEDICATION PRODUCTS IN THE PHARMACEUTICAL INDUSTRY

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Due to a gradually increasing competition on the market of self-medication products the quality itself is no longer sufficient.
In this diploma thesis the specific properties of the self-medication products' advertising are presented. The role of media advertising of switch-medicaments in the company Krka, d. d., has been presented. A review of the promotional campaign concerning the Panatus brand name in Slovenia as the so-called "above the line" part of the promotion is also included. In this way, I have confirmed my assertion that an increased market share of this brand name was undoubtedly provoked by an efficient advertising campaign.
It has been established that Krka's advertising policy differs, within the scope of the OTC programme, depending on the final goal, target group, market competition, environment for our activities, etc. It is the result of clearly formulated market and communication objectives. In the advertising policy all four elements of the marketing management appear, which are the analysis, planning, implementation and control. In the advertising campaign for the Panatus brand name an advantage has been taken using the situation when the medicaments were transferred from the positive to the negative list. Target-aimed advertising policy has succeeded to increase the product's market share.
Apart from affirming the product's brand name, we have strengthened, with a proper advertising policy, the company's good name, which has an influence on the sales promotion and has improved the sales results. This is the final goal of advertising.

3

DIAGNOSTIČNA IN TERAPEVTSKA OBRAVNAVA BOLNIKOV, HOSPITALIZIRANIH ZARADI SRČNEGA POPUŠČANJA

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Srčno popuščanje je pogosto bolezensko stanje s slabo prognozo. Epidemiološke raziskave kažejo na slabo udejanjanje smernic v klinični praksi. V Sloveniji imamo malo podatkov o obravnavi bolnikov s srčnim popuščanjem.
V prospektivni raziskavi smo pregledali bolnišnično dokumentacijo bolnikov, hospitaliziranih v šestih tednih na Interni kliniki Kliničnega centra Ljubljana in Internem oddelku Splošne bolnišnice Murska Sobota ter vključili bolnike, ki so se zdravili zaradi srčnega popuščanja.
Pregledali smo 1035 kartotek in vključili 262 bolnikov (149 moških), starih 72.2±13.1 let. Skupini sta bili primerljivi po starosti in številu bolnikov, trajanju hospitalizacije in smrtnosti. Ob sprejemu je bilo 95% bolnikov v III. in IV. funkcijskem razredu po klasifikaciji NYHA. Ugotovili smo naslednje bolezni: akutni infarkt srca (23%), angino pektoris (30%), arterijsko hipertenzijo (41%), sladkorno bolezen (30%), bolezen dihal (29%) in ledvično odpoved (24%). Elektrokardiogram, rentgenogram srca in pljuč in osnovne laboratorijske preiskave so bile opravljene pri domala vseh bolnikih. Izvid ultrazvočne preiskave srca je imelo 73% bolnikov iz Kliničnega centra in 37% bolnikov iz Splošne bolnišnice. Ob koncu zdravljenja v bolnišnici so bolniki prejemali naslednja zdravila (v odstotkih v Kliničnem centru oz. Splošni bolnišnici): furosemid (65 oz. 80), spironolakton (32 oz. 25), zaviralci angiotenzinske konvertaze (77 oz. 48), zaviralci beta adrenergičnih receptorjev (23 oz. 7), digoksin (38 oz. 73).
Srčno popuščanje je pogosto med hospitaliziranimi bolniki in je najpogosteje posledica ishemične bolezni srca in arterijske hipertenzije. Diagnostična in terapevtska obravnava bolnikov je bila v Kliničnem centru skladnejša s priporočili kardioloških združenj.

DIAGNOSTIC AND THERAPEUTIC TREATMENT OF PATIENTS, HOSPITALISED FOR HEART FAILURE

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Heart failure is common and deadly medical condition. Epidemiological studies showed gap between guidelines and clinical practice. There is a scarcity of heart failure data in Slovenia.
In a prospective comparative study we screened hospital records of patients, discharged from Departments of Internal Medicine in Clinical Centre Ljubljana and General Hospital Murska Sobota during six weeks. Patients with heart failure were included in the study.
Out of 1035 records 262 patients (149 men), aged 72.2±13.1 years, fulfilled the inclusion criteria. Groups were comparable for number, age, hospitalisation time and in-hospital mortality. At admission most patients (95%) were in NYHA functional class III. and IV. They presented with following diseases: acute myocardial infarction (23%), angina pectoris (30%), arterial hypertension (41%), diabetes mellitus (30%), pulmonary disease (29%) and renal failure (24%). Electrocardiogram, chest X-ray and basic laboratory tests were done in almost all patients. Patients from Clinical Centre and General Hospital had echocardiography report in 73% and 37%, respectively. Pharmacologic treatment (in %) in Clinical Centre vs. General Hospital was as follows: furosemide (65 vs. 80), spironolactone (32 vs. 25), angiotensin converting-enzyme inhibitors (77 vs. 48), beta blockers (23 vs. 7) and digoxin (38 vs. 73).
Heart failure is common finding with ischemic heart disease and arterial hypertension as most important aetiologies. Guidelines for diagnostic and therapeutic treatment were better implemented in Clinical Centre.

3

REAKCIJE 3-BROMO-2,2,6,6-TETRAMETILPIPERIDIN-4-ONA S C-NUKLEOFILI

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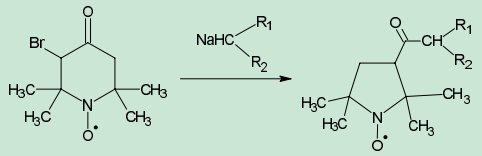
Nitroksidni radikali so pomembno orodje v spektroskopiji elektronske paramagnetne resonance (EPR), kjer se uporabljajo kot spinski označevalci in spinske probe. Pri preučevanju bioloških vzorcev s pomočjo nitroksilnih radikalov je pomembno, da je le-ta odporen na bioredukcijo. Najbolj odporni nitroksidi so petčlenski pirolidinski nitroksidi.
V predstavljenem raziskovalnem delu sem preučevala pogoje pri reakciji 1-oksil-3-bromo--2,2,6,6-tetrametilpiperidin-4-ona (Tempon-a) z različnimi natrijevimi solmi z reaktivno metilensko skupino. Med reakcijo, ki poteka v več stopnjah, pride do Favorskijeve premestitve in redukcije šestčlenskega piperidinskega obroča v petčlenski pirolidinski obroč. Produkti reakcij so nov tip pirolidinskih nitroksidov, opremljeni z novimi funkcionalnimi skupinami, ki dajejo možnosti nadaljnih molekulskih modifikacij za uporabo v EPR študijah različnih sistemov.



V eksperimentalnem delu sem ugotovila, da Favorskijeva premestitev s C-nukleofili poteka, vendar poleg željenih 1-oksil-2,2,5,5-tetrametilpirolidinskih derivatov nastajajo kot stranski produkti tudi diketoni, 1-oksil-2,2,5,5-tetrametilpirolidinski derivati, poteče pa tudi hidroliza estrske vezi v stranski R_2 skupini.

REACTIONS OF 1-OXYL-3-BROMO-4-OXO--2,2,6,6-TETRAMETHYL PIPERIDINE WITH C-NUCLEOPHILES

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Nitroxide radicals are useful tool as spin labels and spin probes in electronic paramagnetic resonance (EPR) spectroscopy. When studying biosamples it is important that stable nitroxyl radicals are used because of bioreduction. The most stable nitroxyl radicals are five-member chain pyrolidine nitroxides.
In this work we described a short and direct synthesis of 1-oxyl-2,2,5,5-tetramethylpyrrolidines from 1-oxyl-3-bromo-4-oxo-2,2,6,6-tetramethylpiperidine (Tempon) as a result of Favorski rearangement. Favorski rearangement itself is successful with C- nucleophiles. However, side products like diketones of 1-oxyl-2,2,5,5-tetramethylpyrrolidines are also obtained and hydrolysis of ester bond in side chain occurs.

3

ŠTUDIJ FIZIKALNIH LASTNOSTI KRISTALNIH MODIFIKACIJ PIROKSIKAMA

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Polimorfizem je pojav, pri katerem ima kemijska spojina v trdnem agregatnem stanju različne kristalne oblike, v tekočem in plinastem stanju pa so identične. Prav različna oblika kristalov je razlog, da imajo polimorfne oblike lahko različne fizikalno-kemijske in termodinamske lastnosti in zato posledično tudi različno biološko uporabnost.
Piroksikam je nesteroidna protivnetna učinkovina, za katero so po podatkih iz literature znani dve polimorfni obliki in solvat v obliki monohidrata. S študijem kristalizacije piroksikama smo ugotovili, da imata izbira topila in hitrost ohlajanja prenasičenih raztopin pomemben vpliv na nastanek polimorfne oblike. Z uporabo različnih analiznih metod (dinamično diferenčno kalorimetrijo-DSC, termogravimetrično analizo-TGA, infrardečo-IR in ramansko spektroskopijo, rentgensko analizo, ^{13}C-NMR trdnih vzorcev in analizo velikosti delcev), ki omogočajo identifikacijo snovi v trdnem agregatnem stanju, smo dokazali, da lahko s kristalizacijo pridobimo tri polimorfne oblike piroksikama in monohidrat. Hitro ohlajanje etanolne raztopine na trdnem CO_2 in sušenje z razprševanjem sta povzročila nastanek nove polimorfne oblike piroksikama. Pri preskusu hitrosti raztapljanja po USP XXIV smo ugotovili, da se najhitreje raztaplja monohidrat piroksikama. Na hitrost raztapljanja imata največji vpliv polimorfna oblika in kot močenja, saj se polimorfna oblika II, ki ima najnižji stični kot, najhitreje raztaplja.
Na osnovi eksperimentalnih rezultatov lahko zaključimo, da je, kljub višji hitrosti raztapljanja polimorfnih oblik II in III, za vgradnjo v trdne farmacevtske oblike zaradi svoje fizikalne stabilnosti najprimernejša polimorfna oblika I.

STUDY OF PHYSICAL PROPERTIES OF PIROXICAM CRYSTAL MODIFICATIONS

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Polymorphism is the condition in which a solid chemical compound exists in more than one crystalline form, although their solutions and vaporous are identical. Differences in the crystalline forms cause differences in physicochemical and thermodynamic properties and consequently bioavailability of polymorphs.
Piroxicam is a nonsteroidal antiinflammatory drug (NSAID) that according to literature exists in two polymorphic forms and as solvate-monohydrate. During study of crystallisation we found out that selection of solvent and rate of cooling supersaturated solutions have an important impact on the crystallisation. Analytical methods such as differential scanning calorimetry (DSC), thermogravimetry (TGA), IR and Raman spectroscopy, X-ray powder diffractometry and solid state ^{13}C-NMR show that with crystallisation we developed three polymorph forms and monohydrate. With very rapid cooling ethanol solution on CO_2 and spray drying we produced new polymorph form of piroxicam. With the dissolution rate by USP XXIV we came to conclusion that the monohydrate had the fastest dissolution rate and that the crystal structure and contact angle had the biggest influence on the dissolution rate. The less stabile form II and form with the lowest contact angle had the fastest dissolution rate.
Due to physical stability of polymorphic form I it was found to be the most suitable form for incorporation in to the dosage forms inspite polymorphic forms II and III shows higher dissolution rate.

3

VPLIV MONOKLONSKIH PROTITELES PROTI KATEPSINU B IN PROTEINAZNIH INHIBITORJEV NA PROLIFERACIJO, INVAZIJO IN ANGIOGENEZO TUMORSKIH CELIC *IN VITRO*

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Lizosomske cisteinske in aspartatne proteinaze katepsini B, L in D sodelujejo pri procesih razvoja in napredovanja raka. Proteolitska aktivnost katepsina B je udeležena pri razgradnji zunajceličnega matriksa, kar omogoča invazijo in metastaziranje tumorskih celic ter ožiljenje tumorjev. Uporaba monoklonskih protiteles, ki inhibirajo aktivnost encimov, predstavlja alternativo naravnim in sintetskim inhibitorjem proteinaz v protitumorski terapiji, saj slednji zaradi nespecifičnega delovanja in toksičnosti *in vivo* niso dali pričakovanih rezultatov.
Z metodo spajanja celic smo pripravili monoklonsko protitelo, ki se veže na epitop v bližini aktivnega mesta katepsina B in inhibira njegovo aktivnost pri razgradnji fluorogenih substratov. Monoklonsko protitelo je v koncentracijah, ki niso vplivale na viabilnost in proliferacijo transformiranih človeških epitelijskih celic dojke MCF-10A neoT, zmanjšalo njihovo invazijo skozi matrigel *in vitro* (47.3 % pri 0.5 µM koncentraciji). V primerjavi z izbranimi proteinaznimi inhibitorji je bolj specifično in zavira invazijo *in vitro* pri nižjih koncentracijah. Med inhibitorji cisteinskih proteinaz je imel največji učinek kokošji cistatin, zatem E-64, medtem ko je bil učinek specifičnega inhibitorja katepsina L CLIK-148 manjši od obeh splošnih inhibitorjev cisteinskih proteinaz. Invazija celic se je zmanjšala tudi v prisotnosti inhibitorjev aspartatnih proteinaz, kar kaže na sodelovanje aktivnih aspartatnih proteinaz pri tumorski invaziji. E-64 je zaviralno vplival tudi na angiogenezo (ožiljenje) v *in vitro* modelu tumorske angiogeneze s celicami HUVEC, kar potrjuje sodelovanje cisteinskih proteinaz pri tem procesu.

THE EFFECTS OF ANTI-CATHEPSIN B MONOCLONAL ANTIBODIES AND PROTEINASE INHIBITORS ON TUMOR CELL PROLIFERATION, INVASION AND ANGIOGENESIS *IN VITRO*

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Lysosomal cysteine and aspartic proteinases cathepsins B, L and D participate in the processes of tumor progression. The proteolytic activity of cathepsin B has been suggested to facilitate degradation of extracellular matrix, leading to invasion, metastasis and tumor angiogenesis. Monoclonal antibodies inhibiting enzyme activity, represent alternative tool towards natural and synthetic proteinase inhibitors in anticancer therapy.
By cell fusion, we prepared monoclonal antibody (MAb) capable to bind an epitope near the active site of cathepsin B and to inhibit *in vitro* its proteolytic activity against fluorogenic substrates. MAb decreased matrigel invasion of transformed human breast epithelial cells MCF-10A neoT *in vitro* (47.3 %, 0.5 µM concentration), whereas no effect on cell viability and proliferation was observed. It is more specific and inhibits the invasion *in vitro* at lower concentrations compared to the selected proteinase inhibitors. Among cysteine proteinase inhibitors chicken cystatin was the most effective one, followed by E-64, whereas for cathepsin L specific inhibitor CLIK-148 showed lower inhibition than both general inhibitors of cysteine proteinases. Tumor cell invasion was decreased also in the presence of aspartic proteinase inhibitors, suggesting that proteolyticaly active aspartic proteinases participate in tumor cell invasion. Additionally, E-64 decreased angiogenesis with HUVEC cells *in vitro*, suggesting that cysteine proteinases participate in this process.

3

NADGRADNJA KONTROLINGA V TRGOVSKEM PODJETJU NA PRIMERU DRUŽBE ERA, D. D., VELENJE

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V magistrskem delu so najprej podane osnovne opredelitve kontrolinga. Kontroling je predstavljen kot samostojno področje v podjetju. Namen kontrolinga je informacijsko - usmerjevalna vloga v smislu priprave kakovostnih, predvsem zgoščenih informacij za poslovno odločanje ter presojanje uspešnosti in učinkovitosti poslovanja. Na osnovi podanih opredelitev je v nadaljevanju predstavljen splošni model kontrolinga. Znotraj modela so podrobno opisane lastnosti in sestavine osnovnih mehanizmov kontrolinga. Pri snovanju splošnega modela sem izhajal iz predpostavke, da so osnovne opredelitve, pristopi in mehanizmi sodobnega kontrolinga v splošni obliki uporabni za podjetja večine panog. Seveda pa posebnosti trgovske panoge in specifične lastnosti podjetja samega vplivajo na način snovanja in izvajanja mehanizmov kontrolinga znotraj podjetja (kontingenčni pristop).
V praktičnem delu naloge je prikazan projekt nadgradnje kontrolinga v družbi ERA, d. d. Najprej je podana vizija in poslanstvo kontrolinga v podjetju. Sledi analiza konkurenčnih dejavnikov v okolju podjetja. V tretjem sklopu projekta je prikazana uporaba predstavljenega splošnega modela pri analizi obstoječega stanja kontrolinga v družbi ERA, d. d. Na podlagi analiz obstoječega procesa planiranja, obstoječih oblik nadzora in obstoječega informacijskega sistema v podjetju sem opredelil lastnosti trenutnih mehanizmov kontrolinga. Opredeljene lastnosti in dodatne analize zunanjega okolja (panoga, tehnologija) in notranjega okolja (organizacijska struktura ter ostali notranji dejavniki) so osnova za oblikovanje predlogov in implementacijo izboljšav posameznih mehanizmov kontrolinga v podjetju.

UPGRADING OF CONTROLLING IN CASE OF THE RETAIL COMPANY ERA, P. LTD. CO., VELENJE

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In the first place the thesis describes fundamental definitions of controlling in the company. Controlling is presented as an independent function within the company. The purpose of controlling is to provide both informational and guiding role in terms of preparing quality, mainly dense information for business decision making and measuring the effectiveness and efficiency of company's performance. According to the fundamental definitions a general model of controlling has been developed in the second part of the thesis. Within the model detailed characteristics of the basic mechanisms of controlling are described. The model is based on the contingency approach. The mentioned approach assumes that specific characteristics of the company need to be considered along with the fundamentals definitions of the controlling when upgrading the controlling in the company. These characteristics come out of the company's external as well as the internal environment. Therefore, the described model can be used in different companies regardless of the branch in which a company operates.
The practical part of this thesis shows a project approach to upgrading of controlling in case of the retail company ERA, P. Ltd. Co. Controlling vision and mission is suggested in the first step of the project. The thesis further talks about analyzing competitive factors in the external environment of the company. In the third step of the project a developed model of controlling has been used for analyzing the current situation of individual mechanisms of the controlling. On the basis of analyses of the planning process, the control process and the information system of the company, characteristics of individual mechanisms have been defined. These characteristics and additional analyses of external (competition, technology) and internal (organizational structure and other factors) environment represent the basis for improvement of individual mechanisms of the controlling in case of ERA company.

3

VPLIV DODATKOV RAZLIČNIH VIROV VLAKNINE V OBROKU Z VELIKIM DELEŽEM MAŠČOB NA OKSIDACIJSKI STRES PRI PRAŠIČIH

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Vpliv prehranske vlaknine (PV) na oksidacijski stres še ni dobro raziskan. V raziskavi na rastočih prašičih smo proučevali, ali je mogoče oksidacijski stres celotnega organizma, povzročen z velikim deležem maščob v obroku zmanjšati z dodatkom PV. 48 kastratov smo vhlevili v kletke, ki omogočajo ločeno zbiranje seča, in jih krmili s krmo, ki je zagotavljala 2,5-kratno kritje vzdrževalnih potreb. Živali smo razdelili v štiri skupine, ki so dobivale enak osnovni krmni obrok, kateremu so bili glede na poskusno skupino izokalorično dodani: v skupini KONT škrob, v skupini OLJE laneno olje, v skupini OLJE+PO laneno olje in pšenični otrobi in v skupini OLJE+OO laneno olje in ovseni otrobi. Stopnjo oksidacijskega stresa in koncentracijo lipidov v krvi smo ovrednotili z meritvami na začetku in koncu 14-dnevnega poskusnega obdobja. S pomočjo HPLC metode smo merili koncentracijo malondialdehida (MDA) v krvni plazmi in seču ter količino dnevno s sečem izločenega MDA. Stopnjo poškodb DNK levkocitov smo ocenili s pomočjo kometnega testa (elektroforeza posameznih celic v gelu). Rezultati kažejo, da je v primerjavi s skupino KONT v skupini OLJE prišlo do statatistično značilno večjega oksidacijskega stresa (večja koncentracija MDA v krvni plazmi, večje izločanje MDA s sečem in večja stopnja poškodb DNK levkocitov) in negativnega vpliva na koncentracijo lipidov v krvi. Dodatek PV v skupinah OLJE+PO in OLJE+OO je v primerjavi s skupino OLJE statistično značilno zmanjšal tako količino s sečem izločenega MDA kot stopnjo poškodb DNK levkocitov. Predvsem dodatek ovsenih otrobov je ugodno vplival na koncentracijo lipidov v krvi. Rezultati raziskave tako kažejo, da dodatek PV učinkovito zmanjša oksidacijski stres, povzročen s prekomernim vnosom maščob.

THE INFLUENCE OF VARIOUS DIETARY FIBERS SOURCES SUPPLEMENTATION ON OXIDATIVE STRESS IN PIGS FED HIGH FAT DIETS

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The influence of dietary fiber (DF) on the oxidative stress of organism is not yet well studied. The aim of research was to evaluate the influence of DF on oxidative stress induced by a high proportion of fat in the diet. 48 young growing castrated male pigs were individually panned in balance cages and fed 2,5 times maintenance requirements. The animals were divided in to four groups which received isocaloric daily rations composed of the equal amount of basic ration supplemented according to the different dietary treatments with: starch (KONT), flex oil (OLJE), flex oil and wheat bran (OLJE+PO), flex oil and oat bran (OLJE+OO). The oxidative stress and the concentration of lipids in the blood were evaluated at the beginning and at the end of the 14 days lasting experiment. The concentration of malondialdehyde (MDA) in blood plasma and urine was measured by HPLC method. The degree of leucocyte DNA damage was measured by comet assay (single cell gel electrophoresis). The experiment confirmed that the high proportion of fat in the diet increases oxidative stress. In comparison to KONT a significant increase in the MDA concentration in blood plasma, significantly higher 24 hour MDA excretion in urine and significantly higher degree of DNA damage in the group OLJE was observed. In comparison to the group OLJE the DF supplementation in both groups significantly reduced the 24 hour MDA excretion in urine and the degree of DNA damage in leucocytes. The beneficial effect of DF supplementation on the blood lipids was particularly pronounced in the oat bran supplemented group. The results of the experiment showed that appropriate proportion of DF in the diet effectively reduces the oxidative stress induced by the high dietary fat intake.

3

SINTEZA IN KONFORMACIJSKA ANALIZA BENZIL (2S)-1-{[(2R/S)-2-METIL-7-NITRO-3-OKSO-3,4-DIHIDRO--2*H*-1,4--BENZOKSAZIN-2-IL]KARBONIL}-2-PIROLIDIN KARBOKSILATA

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V nalogi smo pripravili dva diastereoizomera, benzil (2S)-1-{[(2S)-2-metil-7-nitro-3-okso-3,4-dihidro--2*H*-1,4-benzoksazin-2-il]karbonil}-2-pirolidin karboksilat (**1a**) in benzil (2S)-1-{[(2R)-2-metil-7-nitro-3-okso-3,4-dihidro-*2H*-1,4-benzoksazin-2-il]karbonil}-2-pirolidin karboksilat (**1b**), ki v svoji strukturi vsebujeta prolin in bi lahko bila obetavna sintona v sintezi aktivnih in selektivnih inhibitorjev trombina. Omejitev cis/trans izomerizacije peptidne vezi prolina ali omejitev pirolidinskega obroča v eno samo konformacijo je izredno pomembno za razvoj novih učinkovin. Z metodami moderne 2D NMR spektroskopije smo določili celotni konformaciji obeh spojin. Ugotovili smo, da je diastereoizomer (**1a**) v raztopini DMSO-d_6 v celoti trans izomer peptidne vezi prolina, spojina (**1b**) pa je zmes 83% trans in 17% cis izomera peptidne vezi prolina. Poleg celotne konformacije molekule smo proučili tudi gubanje pirolidinskega obroča prolina in ugotovili, da obroč zavzema konformacijo Cβ endo in konformacijo, ki je med Cδ exo in Cδ exo-N endo preklopljeno obliko.

SYNTHESIS AND CONFORMATIONAL ANALYSIS OF BENZYL (2S)-1-{[(2R/S)-2-METHYL-7-NITRO-3-OXO-3,4-DIHYDRO--*2H*-1,4-BENZOXAZIN- 2-YL]CARBONYL}-2-PYRROLIDINE CARBOXYLATE

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In this work the syntheses of benzyl (2S)-1-{[(2S)-2-methyl-7-nitro-3-oxo-3,4-dihydro-*2H*-1,4-benzoxazine-2-yl]carbonyl}-2-pyrrolidinecarboxylate (**1a**) and benzyl (2S)-1-{[(2R)-2-methyl-7-nitro-3-oxo--3,4-dihydro-*2H*-1,4-benzoxazine-2-yl]carbonyl}-2-pyrrolidinecarboxylate (**1b**) are presented. The cis/trans ratio of the proline peptide bond and concomitant pyrrolidine ring puckering changes in order to exhibit one conformation only is of the highest importance in the development of new active compounds. With the help of modern 2D NMR spectroscopy we determined conformations of both diastereomers and established that the compound (**1a**) is only a trans isomer of the prolin peptide bond, while its diastereoisomer (**1b**) is a mixture of 83% trans and 17% cis form. Studies of the pyrrolidine ring puckering in the solution showed that the most favourable conformations of benzyl (2S)-1-{[(2S)-2-methyl-7-nitro-3-oxo-3,4-dihydro-*2H*--1,4-benzoxazine-2-yl]carbonyl}-2-pyrrolidinecarboxylate (**1a**) are Cβ endo and conformation between Cδ exo and Cδ exo-N endo twist.

3

ANALIZA JEDRNIH SKUPKOV ŽIVČNO-MIŠIČNEGA STIKA V VLAKNIH HITRE IN POČASNE SKELETNE MIŠICE

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V področju živčnomišičnega stika prečno progastega mišičnega vlakna pri sesalcih so jedra bolj zgoščena kot izvensinaptično. Počasna skeletna mišična vlakna so elektromehanično aktivna večji del časa kot hitra vlakna in so tako tudi vzorci frekvenc prenešenih akcijskih potencialov pri obeh tipih vlaken različni, kar bi se lahko odrazilo tudi v različni razporeditvi mišičnih jeder in Schwannovih celic v sinaptičnem področju.
V posamičnih skeletnih mišičnih vlaknih, ki smo jih izolirali iz m.extensor digitorum longus (hitra vlakna) in m. soleus (počasna vlakna), smo obarvali jedra s pomočjo fluorescenčnega barvila "SYTOX zeleno". Živčnomišični stik smo v teh vlaknih obarvali s pomočjo α-bungarotoksina, označenega z rodaminom. Ustrezna morfometrična merjenja razporejenosti jeder smo opravili na konfokalnem mikroskopu
Dobljeni rezultati so nas pripeljali do naslednjih zaključkov: 1) V sinaptičnem jedrnem skupku odraslega počasnega skeletnega mišičnega vlakna je 7,15±2,37 (n=20) mišičnih jeder, medtem ko je v sinaptičnem jedrnem skupku hitrega vlakna teh jeder 4,60±0,75 (n=20); 2) Korelacija med sinaptično površino živčnomišičnega stika in številom jeder, ki pripadajo Schwannovim celicam, je bila v hitrem vlaknu višja (KK=0,4) kot v počasnem (KK =0,07), a po naši oceni še vedno premajhna za potrditev naše druge hipoteze; 3) Maksimalna razdalja v mišičnem vlaknu, do katere so še vidni znaki delovanja živčnih trofičnih dejavnikov, in ki smo jo ocenili z merjenjem razdalje med robom živčnomišičnega stika in prvim zunajsinaptičnim jedrom, znaša za hitra skeletna mišična vlakna 25,5±9,3 µm, (n=20) za počasna pa 26,9±7,4 µm (n=20).

ANALYSIS OF SYNAPTIC NUCLEAR CLUSTERS IN ISOLATED FIBERS OF FAST AND SLOW SKELETAL MUSCLE

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Myonuclei of mamalian skeletal muscle fiber are packed at much higher density in the neuromuscular junction region than in the extrajunctional parts of the fiber. Slow muscle fibers are electromechanically active for longer time periods than fast fibers and the frequency patterns of action potentials are different in both fiber types. This difference might reflect in the distribution of synaptic myonculei and the arrangement of synaptic Schwann cells.
Nuclei in single muscle fibers of m.extensor digitorum longus (fast) and m.soleus (slow) were labelled with "SYTOX green" nuclear acid stain and the endplates with rhodamine-tagged α-bungarotoxin. Images obtained by confocal microscopy were morphometircally analysed
There are 7,15±2,37 (n= 20) synaptic myonuclei in slow muscle fiber and 4,60±0,75 (n=20) synaptic myonuclei in fast muscle fiber; The correlation coefficient between the area of the neuromuscular junction and the number of Schwann cell nuclei was higher in fast fibers (KK=0,4) than in slow fibers (KK=0,07), but to our opinion still too low to confirm our second hypothesis; 3) Maximal distance of the action of neuronal trophic factors was estimated by measuring the distance between the border of the neuromuscular junction and the first extrasynaptic nucleus. It was found to be 25,5±9,3 µm, (n=20) in fast fiber type and 26,9±7,4 µm (n=20) in slow fiber type.

3

IZOLACIJA IN OPIS LAKAZ PRI NARAVNIH IZOLATIH BAKTERIJ RODU *BACILLUS*

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Lakaze (benzendiol:kisik:oksidoreduktaze, EC 1.10.3.2) so raznolika skupina multi-bakrovih proteinov, ki katalizirajo oksidacije različnih aromatskih spojin. Zaradi široke substratne specifičnosti imajo velike možnosti za uporabo v biotehnologiji. Iz vzorcev, ki smo jih nabrali na različnih mestih po Sloveniji, kjer smo pričakovali povečano koncentracijo polifenolnih spojin, smo želeli izolirati bakterije z lakazno aktivnostjo. Na hranilnem agarju smo osamili bakterijske seve in odbrali tiste z dobro izraženo lakazno aktivnostjo. V ta namen smo sestavili kompleksno agarsko gojišče s škrobom in sojino moko. Pri zraslih kolonijah smo testirali lakazno aktivnost s specifičnimi substrati (2,6 - dimetoksifenol, siringaldazin, ABTS). Izbrali smo 6 sevov, ki so razgrajevali vse tri substrate. Identifikacija je pokazala, da pripadajo rodu *Bacillus*. Pri vseh sevih smo dokazali prisotnost lakaze na sporah. Izvleček encima najaktivnejšega seva VM-1 smo pridobili s kombinacijo mehanske sile in kemične obdelave spor z mešanico 7M uree + 1%SDS + 0,1 M NaOH. Pri čiščenju encima smo uporabili dializo, koncentracijsko centrifugiranje skozi membranske filtre, gelsko filtracijo in ionsko-izmenjevalno kromatografijo. S SDS-PAGE elektroforezo smo ugotovili, da je molekulska masa encima 34 kDa. Delno izoliranemu encimu in encimu na sporah smo s substratom ABTS spektrofotometrično kvantitativno določili temperaturni in pH optimum ter vpliv nekaterih lakaznih inhibitorjev. Encimska aktivnost je bila največja pri temperaturi 85°C in pH = 4-5. Lakaza izolata *B. licheniformis*, sev VM-1 je ena prvih delno izoliranih nativnih lakaz pri bakterijah. Menimo, da sta izbrani izolat in njegova delno izolirana lakaza obetavna za biotehnološko uporabo.

ISOLATION AND CHARACTERIZATION OF LACCASE FROM BACTERIAL ISOLATES BELONGING TO THE GENUS *BACILLUS*

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Laccases (benzenediol:oxygen:oxidoreductases) are diverse group of multi-copper proteins that catalyse the oxidation of a variety of aromatic compounds. Because of their broad substrate specificity, laccases harbor great biotechnological potencial. The isolation of bacteria possessing lacase activity was carried out from samples collected on potentially polyphenol reach sites all over Slovenia. Pure cultures were isolated from nutrient agar plates and those with highest laccase activity selected out. To induce maximum hidrolytic potential a complex soy and starch based medium was formulated. Microbial colonies were screened for laccase activity on specific substrates for laccases (syringaldazine, 2,6-di-metoxi-phenol and ABTS). We selected 6 isolates that were able to degrade all three substrates. The strains were identified as members of *Bacillus sp*. The laccase activity was detected on spores by selected strains. Enzyme extraction from the most active strain VM-1 was successful with a chemical mixture U-MIX (7M urea, 1%SDS, 0.1 M NaOH) in combination with mechanical treatment. Laccase was purified by dialysis, concentration, by centrifugation in tubes with membrane filters, gel filtration and ion exchange chromatography. The size of laccase (34 kDa) was determinated by SDS-PAGE electrophoresis. The temperature and pH optimum and the effects of selected laccase inhibitors were determined for the partially isolated enzyme and the enzyme on spores. The laccase showed best activity at temperature 85°C and pH = 4-5. The laccase of *B. licheniformis*, strain VM-1 is one of the first partially isolated native bacterial laccases. The strain VM-1 itself and its laccase have promising biotechnological characteristics.

3

ANALIZA VARIABILNOSTI ODZIVOV SKELETNIH MIŠIC NA ELEKTRIČNO STIMULACIJO

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Tenziomiografija (TMG) je neinvazivna, selektivna in univerzalna metoda spremljanja kontraktilnih lastnosti skeletnih mišic. Uporabnost metode TMG je velika na področjih športa in medicine.
V magistrski nalogi je bila podana analiza variabilnosti metode TMG. Parametri, ki vplivajo na variabilnost metode so bili izločeni in analizirani njihovi vplivi na variabilnost metode. Bipolarna električna stimulacija se sestoji iz posamičnega električnega dražljaja, z nastavljivo amplitudo, širino in časa med zaporednimi dražljaji. Širina električnega dražljaja ne vpliva na rezultat metode. Dolžina časovnega intervala med zaporednimi električnimi dražljaji naj bo večja od 10s, da se izloči vpliv zaporedne električne stimulacije na rezultat metode. Višja amplituda električnega dražljaja, pomeni več aktiviranih motoričnih enot mišice in s tem celovitejša slika mišice.
Parametri senzorja in merilnega okolja so pritisk senzorja na mišico, kot postavitve senzorja, izbira merilnega mesta in relativna dolžina mišice med izvajanjem meritev. Pritisk senzorja na mišico se regulirata z površino tipala in koeficientom povratne vzmeti v senzorju. Pritisk in kot postavitve senzorja minimalno vplivata na rezultat meritve. Dolžina mišice se spreminja s spreminjanjem kota v sklepu. Kot v sklepu značilno vpliva na rezultat metode, zato ga je potrebno standardizirati za manjšo variabilnost metode. Metoda TMG je bila nadgrajena v metodo večkanalnega TMG s pomočjo katere smo ugotovili področja na mišici, kjer je variabilnost metode manjša od 5%.
Pri analizi variabilnosti so bile predlagane standardizacije parametrov variabilnosti, merilec se jih mora zavedati in vključno s svojimi bogatimi izkušnjami minimizirati variabilnost metode TMG.

ANALYSIS OF VARIABILITY OF SKELETAL MUSCLE RESPONSES ON ELECTRICAL STIMULATION

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Tensiomyography (TMG) is noninvasive, selective and universal measuring method for detection of contractile properties of skeletal muscles. Relevance of TMG method is of great importance in fields of sport and medicine.
In this work variability of TMG method was studied. Parameters, which have impact on variability of TMG method, were defined and analyzed. Bipolar electrical stimulation consists of single stimulus with adjustable amplitude, stimulus width and time interval between electrical stimuli. Analysis shows that stimulus width has no impact on TMG response. The time interval duration must be at least 10s in order to avoid its impact on measuring results. Higher amplitude of electrical stimulus means more activated motor units and this enables the TMG response to reflect the activity of the whole muscle.
Parameters of sensor and measuring environment were pressure of sensor tip on muscle, angle between sensor and muscle, measuring point and relative muscle length during measuring protocol. Pressure of sensor tip is regulated by coefficient of the string (comprised in sensor) and size of sensor tip. The pressure and angle have no impact on variability of TMG measuring results. Muscle length depends on angle in the joint about which the muscle is attached. Results show that changing the angle does impact TMG response, therefore the position of measured person has to be standardized in order to minimize the variability. Development of multi-channel TMG method as an upgrade of single-channel TMG method was necessary to define the measuring area within which the measuring point should be selected (to ensure minimum variability).
According to results of presented analyses, standardizations for studied parameters were suggested. Considering these standardizations the measurer with his experiences can contribute to minimization of the variability of the TMG method, which was established less then 5%.

3

SINTEZE HETEROCIKLIČNIH SISTEMOV IZ DIAMINOMALEONITRILA

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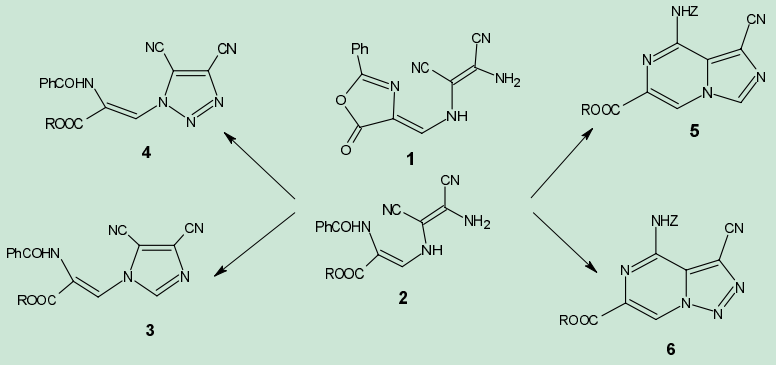
V sintezni heterociklični kemiji imajo pomembno vlogo reagenti, ki jih uporabljamo za uvedbo določenih funkcionalnih skupin oz. delov molekul, ki so primerni za nadaljnje pretvorbe. Eden od reagentov takšne vrste je diaminomaleonitril.
V tej raziskovalni nalogi sem raziskal pretvorbe novih N-monosubstituiranih derivatov diaminomaleonitrila **1** in **2** v derivate imidazola **3**, 1,2,3-triazola **4**, imidazo[1,5-*a*]pirazina **5**, [1,2,3]triazolo[1,5-*a*]pirazina **6** in v druge heterociklične spojine ter nadaljnje pretvorbe omenjenih produktov.[1-2]
Strukture nastalih spojin in njihovih tavtomerov v raztopini sem določil z uporabo različnih 2D NMR eksperimentov. V ključnih primerih smo potrdili strukture produktov z rentgensko strukturno analizo.



THE SYNTHESIS OF HETEROCYCLIC SYSTEMS STARTING FROM DIAMINOMALEONITRILE

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Much synthetic heterocyclic organic chemistry involves specially designed reagents which are readily generated and then used to provide molecules with built-in functional moieties for further transformations. Important example of such reagent is diaminomaleonitrile.
In this research the transformations of novel N-substituted diaminomaleonitrile derivatives **1** and **2** into imidazole **3**, 1,2,3-triazole **4**, imidazo[1,5-*a*]pyrazine **5**, [1,2,3]triazolo[1,5-*a*] pyrazine **6** derivatives and other heterocyclic compounds were studied. Further transformations of products were also under investigations.[1-2]
Structures of products and their tautomers in solution were determinated by application of different 2D NMR experiments and were confirmed in key cases by X-ray spectroscopy.

1. Trček, T., Meden, A., Verček, B., *Synlett*. **2000**, 10, 1458.
2. Jug, T., Polak, M., Trček, T., Verček, B., *Heterocycles*, in press.

3

SINTEZA IN PRETVORBE POLISUBSTITIRANIH ARIL ETILENSKIH SPOJIN

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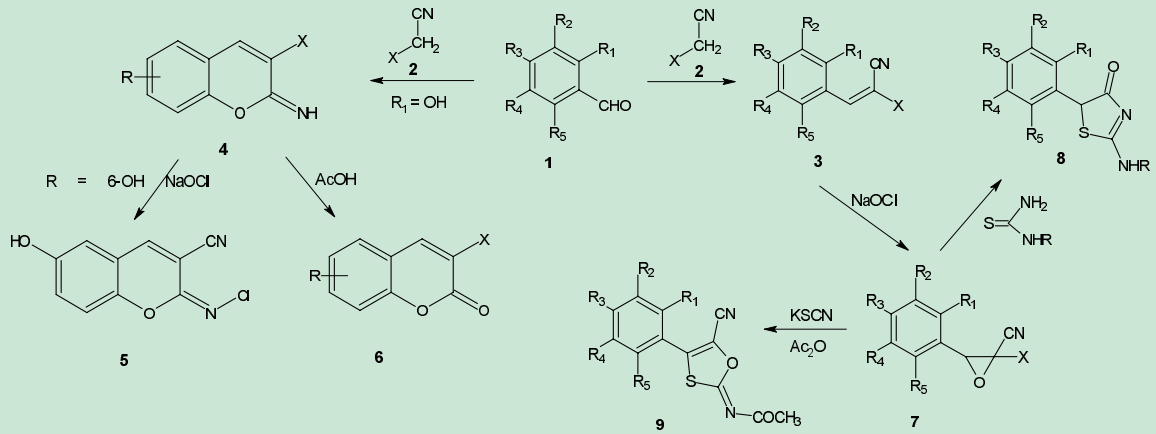
Knoevenaglova kondenzacija metoksi in hidroksi aril aldehidov **1** s spojinami z aktivirano metilensko skupino **2** je vodila do polisubstituiranih aril etilenskih spojin **3**. V nekaterih primerih je prišlo do nastanka bicikličnih analogov **4** (benzopiran-2-iminov) kot posledica nukleofilne adicije orto substituirane hidroksilne skupine fenilnega obroča na ciano skupino stranske vinilne verige. S Knoevenaglovo reakcijo sintetizirane spojine so potencialna antiproliferativna sredstva. Benzopiran-2-imine smo hidrolizirali do kumarinskih derivatov **6**. 6-Hidroksi-2-(N-kloro-imino)-*2H*-3-kromenkarbonitril **5** smo izolirali iz 6-hidroksi-2-imino-*2H*-3-kromenkarbonitrila ob prisotnosti natrijevega klorata (I). Ciano substituirane etilenske spojine smo z natrijevim kloratom (I) pretvorili do ustreznih oksiranskih derivatov **7**, ki so zanimive izhodne spojine. Na oksiranih smo izvedli reakcije z različnimi dušikovimi binukleofili, kakor tudi tiocianatnim ionom. Na ta način smo pripravili tiazolne **8** in oksatiolne **9** derivate.



SYNTHESIS AND TRANSFORMATIONS OF POLYSUBSTITUTED ARYL ETHYLENIC COMPOUNDS

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Knoevenagel condensation of methoxy and hydroxy aryl aldehydes **1** with activated methylenic group **2** led to polysubstituted aryl ethylenic compounds **3**. The bicyclic analogues **4** (benzopyran-2-imines) can also be isolated as a consequence of nucleophylic addition of orto phenyl's hydroxy group to cyano group on the side vinyl chain. Compounds synthesised with the Knoevenagel reaction are potential antiproliferative agents. Benzopyran-2-imines **4** have been hydrolised to coumarine derivatives **6**. In the presence of sodium chlorate (I) 6-hydroxy-2-(N-chloro-imino)-*2H*-3-chromencarbonitrile **5** was obtained from 6-hydroxy-2-imino-*2H*-3-chromencarbonitrile. Oxirane derivatives **7** can be synthesised by the reaction of cyano substituted ethylenic compounds with sodium chlorate (I). Reactions of oxiranes with different nitrogen nucleophiles and tiocyanate ion led to thiazole **8** and oxatiole **9** derivatives.

3

PRIMERJALNA ŠTUDIJA IZDELAVE PELET V ROTORSKEM GRANULATORJU IN HITREM MEŠALNIKU

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Pelete so kroglasti aglomerati, sestavljeni iz zdravilne učinkovine in pomožnih snovi. Z različnimi tehnologijami lahko izdelamo ogrodne ali obložene pelete. V okviru raziskovalnega dela smo pripravili ogrodne pelete s tremi različno topnimi učinkovinami. Osredotočili smo se na primerjavo dveh tehnologij izdelave pelet: v rotorskem granulatorju in hitrem mešalniku, ob dveh načinih dodajanja sredstva za aglomeracijo. V prvem primeru smo kot sredstvo za aglomeracijo dodali raztopino hidroksipropil celuloze, v drugem demineralizirano vodo. Spremljali smo izdelavo pelet in proučevali spremenljivke, ki so kakorkoli vplivale na nastanek aglomeratov. Ukvarjali smo se s problematiko lepljenja peletne mase na steno posode hitrega mešalnika, oziroma komoro rotorskega granulatorja.
Med izdelavo pelet in kasnejšim vrednotenjem le-teh smo ugotovili, da sta tehnologiji priprave pelet v rotorskem granulatorju in hitrem mešalniku primerljivi. Pelete imajo podobne fizikalnotehnološke lastnosti, tudi statistična obdelava je pokazala primerljivost peletnih vzorcev posameznih serij. Izdelava pelet v hitrem mešalniku je bila enostavnejša, vendar so bile izdelane pelete bolj različnih oblik in velikosti, njihove površine manj gladke. Sproščanje učinkovine iz teh pelet se ni razlikovalo od sproščanja učinkovine iz pelet, ki smo jih izdelali v rotorskem granulatorju. S statistično primerjavo profilov sproščanja smo ugotovili, da obstaja podobnost pri enako velikih frakcijah pelet, pripravljenih po obeh tehnologijah izdelave, z obema načinoma dodajanja tekočine za aglomeracijo.

COMPARISON STUDY OF PELLET PRODUCTION IN ROTOR GRANULATOR AND HIGH-SHEAR MIXER

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Pellets are spherical agglomerates containing active ingredient and various excipients. In experimental part of our research work we prepared matrix pellets with three active ingredients that differed in solubility. There are numerous technologies that can be used for preparing matrix pellets. We focused on two of them: the direct rotor pelletization and high sheer mixer pelletization with implementing a binder either as dry substance or in a solution. Monitoring all variables that influenced pelletization and by using statistical method we found these two technologies are comparable as they provide pellets with similar physico-technological properties. Statistical evaluation of dissolution profiles of pellets of the same diameter range showed similarity of the two technologies either is a binder added as dry substance or in a solution.

3

ANALIZA TRGA V NUTRICEVTSKI DEJAVNOSTI

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Nutricevtika je usmerjena na razvoj izdelkov, ki tako preventivno kot kurativno vplivajo na splošno zdravje. V strukturah farmacevtskih in prehrambenih podjetij se nutricevtska dejavnost hitro širi.
V raziskovalni nalogi sem preučil dejavnike makrookolja z modelom W. Gilesa ter dejavnike privlačnosti panoge s Porterjevim modelom.
Model W. Gilesa je pokazal naklonjenost makrookolja za razvoj nutricevtike ter opozoril na nekatere dejavnike, ki ga zavirajo. To so predvsem neusklajenost regulative v vseh državah, ukrepi vlad in pomen nizkih dohodkov v manj razvitih državah. Nutricevtika je namreč visoko kapitalno in razvojno raziskovalno intenzivna panoga, vendar bistveno manj kot farmacevtika.
Porterjev model je pokazal visoko privlačnost panoge z vidika nizkega rivalstva podjetij znotraj panoge. Ovire za vstop v panogo so visoke zaradi neurejene regulative. Nevarnost nadomestkov je v večini segmentov še nizka, kar je posledica "mladosti" panoge. Pogajalska moč kupcev je nizka, kar je posledica masovnega trga in visoke osveščenosti zdravega prehranjevanja. Kupci nutricevtskih izdelkov posvečajo večjo pozornost kakovosti kot nizkim cenam. Moč dobaviteljev je nizka.
Slovenski trg sledi trendnim spremembam v razvitem svetu. Največji domači proizvajalci so Krka, Lek ter večje mlekarne.
Telefonska anketa slovenskih potrošnikov nutricevtskih izdelkov je pokazala visoko osveščenost zdravega prehranjevanja. Najbolj zaželeni so probiotični mlečni izdelki ter pšenični izdelki. Visok pomen daje slovenski kupec kvalitetnim sestavinam, okusu in aromi ter ceni.
Analiza je pokazala celovito oceno panoge (v svetu in pri nas), ki je zelo obetajoča. Skupna ocena na lestvici (0 - 1) znaša za tekoče obdobje 0.65 (dobro) in za prihodnje 0.75 (zelo dobro).

ANALYSIS OF NUTRACEUTICAL MARKET

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Nutraceutical industry has been oriented towards development of products which have preventive and curative effect on general health. Major players in it are pharmaceutical and nutrition companies.
Research paper examines characteristics of environment using W. Giles model and competitive advantages of industry using M. Porter's model.
Analysis of macroeconomic environment has revealed that there exist more positive than negative global trends that affect the industry. Main trends that interfere with the development are slow regulatory harmonization, bad government involvement and low levels of GDP p.c. in less developed countries. Nutraceutical industry is high capital and R&D intensive industry.
The competition in nutraceutical industry is still low due to its high growth and relative "youth". Barriers to entry are high due to slow harmonization of regulations. Substitutes don't exist yet, but some functional food products present a threat to normal food products. Consumer's demands are more on high quality than on low prices of the products. The supplier power is low.
The telephone questionnaire revealed that average Slovenian consumer highly values healthy nutrition. Most important is the quality of ingredients followed by favorable taste, aroma and price. Most evident is the lack of cooperation between companies and schools, hospitals etc.
The complete market analysis gives the nutraceutical industry (on a scale 0 - 1) for today a grade of 0.65 (good) and for the near future a grade of 0.75 (very good).

3

ZMANJŠEVANJE PORABE VODE V PROCESNIH INDUSTRIJAH

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V nalogi smo predstavili razširjen in kombiniran celostni, sistematični pristop zmanjševanja porabe vode v procesnih industrijah, upoštevajoč okoljevarstvene, organizacijske in stroškovne vidike. Prikazali smo tudi pregled optimirnih metod zmanjševanja porabe vode, ki temeljijo na vodnem uščipu in z njim povezanih konceptualnih metod ter metod matematičnega programiranja. Navedli smo vzroke za njihovo omejeno uporabnost. Razviti sistematični pristop smo preizkusili na primerih zmanjševanja porabe vode v tekstilni tovarni in v proizvodnji pridobivanja sladkorja. Analize so pokazale, da je pristop uporaben in omogoča zmanjšanje porabe vode in nastajanja odpadne vode.
Prihranki ponovne uporabe vode pri majhnih porabnikih, v našem primeru v tekstilni tovarni, so manjši v primerjavi z večjimi porabniki vode. Z ukrepi zmanjševanja porabe vode v tekstilni tovarni je možno znižati porabo vode za 10.380 m^3/a, kar predstavlja 12,6 % prihranek celotne porabe vode v tovarni. Z upoštevanjem vgradnje rabljene opreme in cen vode s takso za leto 2001 znaša neto sedanja vrednost predlaganih ukrepov 2.404.000 SIT.
Pri večjem porabniku vode, v našem primeru v proizvodnji sladkorja, je ekonomski izid ugodnejši. Tako je možno z ukrepi zmanjševanja porabe vode v proizvodnji sladkorja znižati porabo vode za 54,23 m^3/h v času trajanja kampanje, kar predstavlja 69 % prihranek celotne porabe vode. Neto sedanja vrednost je 33.560.000 SIT, vračilni rok naložbe je 5 dni. Višji masni delež prihranka vode v primerjavi s tekstilno industrijo izhaja iz narave samega procesa in širših možnosti ponovne uporabe vode zaradi številnih porabnikov vode.
Pričakovano naraščanje cen vode in pripadajoče takse v prihodnosti (v zadnji dveh letih za okrog 100 %) in zniževanje obrestnih mer zaradi vstopa v Evropsko unijo bosta dodatno izboljšala ekonomski izid predlaganih projektov in tako upravičila njihovo izvedbo.

WATER MINIMIZATION IN PROCESS INDUSTRIES

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In this work, the extended and combined systematic approach for water reuse in process industries is presented. Environmental, organizational and economic aspects of water reuse were taken into consideration. A review of design procedures for optimal water reuse (methods, based on water pinch method or related to it, and mathematical programming methods) is presented. The reasons for their limited use are given. The systematic approach developed for water reuse was tested with two case studies, in a textile industry and sugar production. The results have shown the applicability of the systematic approach, giving the possibilities of reducing freshwater consumption on one hand and wastewater generation on the other one.
In the case of small water consumer, textile mill in our case, the savings of water reuse are smaller in comparison to larger water consumer. The options of water minimization proposed in the textile plant could lower the fresh water consumption by 10,380 m^3/a, reducing the total volume of fresh water used by 12,6 %. With regard to the usage of second hand equipment and water prices including tax for the year 2001 the options proposed result in net present value of 2,404,000 SIT.
The economical outcome of water reuse project is advantageous in the case of larger water consumer, sugar mill in our case. The options of water minimization proposed in the sugar production could lower the fresh water consumption by 54,23 m^3/h, reducing the total volume of water used by 69 % with the payback period of 5 days and net present value of 33,560,000 SIT.
The economic outcome will be improved in the future because of the growing water price and taxes as well as the expected lowering of interest rates. This will make the investments more attractive.

3

DOKAZOVANJE VIRUSA SLINAVKE IN PARKLJEVKE Z METODO POLIMERAZNE VERIŽNE REAKCIJE

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V tem delu smo predstavili specifično, hitro in občutljivo metodo dokazovanja vSIP, ki temelji na pomnoževanju RNK virusa slinavke in parkljevke (vSIP) z reverzno transkripcijo in polimerazno verižno reakcijo (RT-PCR). Optimizirali smo metodo, ki nam omogoča izvajanje reverzne transkripcije in pomnoževanje izbranega odseka virusnega genoma v eni stopnji. Vse reagente in encime, tako tiste za reverzno transkripcijo kot tudi tiste za pomnoževanje, dodamo izolirani virusni RNK naenkrat in izvedemo RT-PCR v termopomnoževalniku. Med obema stopnjama tako ni potrebno odpirati epruvet in dodajati reagentov. Tako prihranimo na času in zmanjšamo možnost kontaminacije med vzorci, do katere bi lahko prišlo pri vmesnem odpiranju epruvet in dodajanju reagentov.
Dobljeni produkt RT-PCR smo dokazovali z elektroforezo v agaroznem gelu in s hibridizacijo s specifično oligonukleotidno sondo v mikrotiterskih ploščah (PCR-ELISA).
Specifičnost in občutljivost metode PCR-ELISA smo primerjali z metodo Česmi-nested« PCR, pri kateri produkt RT-PCR ponovno pomnožujemo z notranjimi začetnimi oligonukleotidi.
Začetne oligonukleotide smo izbrali na dveh različnih odsekih virusnega genoma, na genu za polimerazo RNK in na genu za strukturni protein VP1. Z enotnim parom začetnih oligonukleotidov smo pomnožili odsek gena za polimerazo RNK pri vseh sedmih serotipih vSIP. Pri pomnoževanju gena za VP1 pa smo morali uporabiti dva različna para začetnih oligonukleptidov. Z enim parom smo dokazali serotipe A, O, C in Asia 1, z drugim pa viruse iz skupine SAT.

DETERMINATION OF FOOT AND MOUTH DISEASE VIRUS BY THE METHOD OF POLIMERASE CHAIN REACTION

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Recent work represents a sensitive and rapid detection method based on specific amplification of the FMDV RNA by reverse transcription and polimerase chain reaction (RT-PCR). The optimised RT-PCR method enables the reverse transcription and amplification of selected part of viral genome in one step. All reagents and enzymes for RT and PCR are added to isolated viral RNA and the amplification is performed in thermocycler. The need to open the tubes and to add reagents between the RT and the PCR step is eliminated. The possibility of contamination due to reaction tubes opening after initial RT is minimised and the hands-on work required is reduced as well.
The obtained RT-PCR products were analyzed by agarose gel electrophoresis and by microplate hybridisation using specific oligonucleotide probe (PCR-ELISA).
The specificity and sensitivity of RT-PCR method were compared by semi-nested PCR, which enabled additional amplification with internal primers.
The oligonucleotide primers were selected on two different genome regious coding for the RNA polymerase and for the structural protein VP1. The same primer pair successfully amplified genome fragment on the RNA polymerase gene of all seven FMDV serotypes. For the VP1 gene amplification two different sets of primers were used. The first primer pair detected FMDV serotypes A, O, C and Asia1 and the second one viruses from the group SAT.

3

BIOLOŠKA OCENA ONESNAŽENOSTI REKE KRKE NA OBMOČJU OBČINE NOVO MESTO

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Raziskovalna naloga zajema podatke, zbrane na podlagi dveh odvzemov vzorcev rečne podlage reke Krke na štirih lokacijah v občini Novo mesto: Straža (Govejek), Ragov log, Mačkovec in Otočec.
Na podlagi medsebojne primerjave vzorcev sva ugotavljali stopnjo čistosti oziroma onesnaženosti vode na odvzemnih mestih v maju in avgustu.
Kriterij je bilo število in vrsta tam živečih bentoških organizmov, ki so biološki indikatorji in kažejo stopnjo onesnaženja vode, katero sva po pregledu vseh vzorcev izračunali po znani formuli.
Iskali sva vzroke za manjšajočo se stopnjo onesnaženosti.
Dobljene podatke sva primerjali s stopnjami onesnaženosti na tem področju Krke, ki so bile določene v dveh podobnih raziskavah iz preteklih let (1985 in 1993).

BIOLOGICAL EVALUATION OF POLLUTION OF THE KRKA RIVER IN THE AREA OF NOVO MESTO COMMUNITY

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This research paper is based on the data acquired by analysis of two samples from the bottom of the river Krka. They were taken at four different locations in the Novo mesto community: Straža (Govejek), Ragov Log, Mačkovec and Otočec.
The pollution of water, taken from the chosen observation spots in May and August this year, has been established by comparison of the samples.
We focused on the number and the variety of there living benthos organisms which function as biological indicators: they point out the degree of pollution of the water. After the complete examination of the samples, the degree of pollution was calculated with the help of well-known formula.
In our task, we searched for causes of decreasing degree of pollution.
We compared the acquired figures with those from two similar research projects made on this area of the river Krka in years 1985 and 1993.

3

VPLIV BAKRA NA RAST IN RAZVOJ RASTLIN V TKIVNI KULTURI IN SUBSTRATU

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V raziskovalni nalogi smo preučevali vpliv različnih koncentracij bakra na rast in razvoj krompirja *(Solanum tuberosum)* in surfinije *(Petunium × Petunium) in vitro* ter graha *(Pisum sativum)*, jarega ječmena (Hordeum vulgare) in oljne redkve (Raphanus sativus) *in vivo*. Rastline smo gojili pri različnih koncentracijah bakra. Spremljali smo višino poganjkov, število korenin in nodijev *in vitro* ter odstotek kalitve semen *in vivo*. Ugotovili smo, da baker v zakonsko priporočenih mejnih, opozorilnih in kritičnih koncentracijah ne vpliva na rast in razvoj rastlin. Do fitotoksičnih vplivov pride samo v primeru sočasnega znižanja pH vrednosti gojišča oz. substrata ali ekstremno visokih koncentracijah, kot je 6140 mg Cu/kg suhega substrata. Fitotoksični vpliv bakra se kaže v zakrneli rasti in klorozi rastlin *in vitro* ter slabši kalitvi *in vivo*.
Za natančnejšo določitev mejne toksične koncentracije bakra bodo potrebne nadaljnje raziskave z drugimi koncentracijami in oblikami bakra.

THE INFLUENCE OF COPPER ON PLANT GROWTH AND DEVELOPMENT *IN VITRO* AND *IN VIVO*

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In our research the influence of copper on growth and development of potato *(Solanum tuberosum)* and surfinia *(Petunium × Petunium) in vitro* and peas *(Pisum sativum)*, barley *(Hordeum vulgare)* and oil radish *(Raphanus sativus) in vivo* was examined. Plants were grown in medium and substratum with different concentrations of added copper. We observed the height of plants, the number of roots and nodium *in vitro* and the germination of seeds *in vivo*. We discovered that copper in offically recommended limitary, warning and critical concentrations doesn't affect growth and development of plants. Copper is fitotoxic only at lower pH in medium or substratum and at extremly high concentrations (6140 mg Cu/kg of dry substratum). Symptoms of toxicity of copper are chlorosis and inhibition of growth *in vitro* and poorly germination *in vivo*.
Supplemental researches with other concentrations and forms of copper will be needed for more accurate determination of the toxic limits of copper.

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GALVANSKI TOKOVI V USTIH

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Pojav galvanskih tokov je znan že dalj časa. Poznavanje te problematike je zelo pomembno področje, saj se v vseh strokah uporablja kovine in zlitine. Za nastanek galvanskih tokov potrebujemo le dve kovini ter elektrolit. V nekaterih primerih je pojav galvanskih tokov koristen, v drugih pa ne. Mi smo se omejili predvsem na tiste manj prijetne, ki se pojavljajo v ustni votlini.
V teoretičnem delu raziskovalne naloge smo poglobili naše znanje o galvanskih tokovih na splošno ter tudi o galvanskih tokovih v ustni votlini. V anketi smo povprašali bližnje stomatologe o njihovih izkušnjah s to problematiko.
Praktični del smo izvajali v zobnem laboratoriju Milene Simončič, kjer smo merili razliko električnih potencialov med različnimi protetičnimi materiali, potopljenimi v slino. V zasebni ordinaciji stomatologinje dr. Alenke Nikič pa nam je bil razložen marsikateri problem, s katerim smo se soočili.

STRAINS OF GALVANIC CELL IN MOUTH

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Knowledge of galvanic cells' problem is a very important area because metals and alloys are used on many fields of work. For the beginning of the strain of galvanic cell only two metals and an electrolyte are needed. In some cases the appearance of the strains of galvanic cell is useful, in others they are not. We limited our research mainly to those less pleasant cases which appear in the oral route.
At the theoretical part of our research we learned about the galvanic cell in general and also about that in oral route. In the inquiry we asked some local dentists about their experience in this matter.
The practical part was performed in the dental lab of Milena Simončič. We measured the difference of electric potential between different protetic materials, sunk into saliva. In the private ordination of the dentist Alenka Nikič a lot of problems we faced become clear.

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