KRKA

32. Krkine nagrade

32nd Krka Prizes

Novo mesto, Slovenija, 24.-25. oktober 2002

12. mednarodni simpozij
12th International Symposium

www.krka.si/krkine-nagrade
www.krka.si/krka-prizes

Častni odbor 32. Krkinih nagrad

Miloš Kovačič, predsednik uprave in generalni direktor Krke, predsednik
Boštjan Žekš, predsednik Slovenske akademije znanosti in umetnosti
Jože Mencinger, rektor Univerze v Ljubljani
Željko Knez, v. d. rektorja Univerze v Mariboru
Andreja Čufar, predsednica Slovenskega farmacevtskega društva
Venčeslav Kaučič, predsednik Slovenskega kemijskega društva
Pavel Poredoš, predsednik Zdravniškega društva Slovenije

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

Znanstveni odbor 12. mednarodnega simpozija

Miha Japelj, predsednik
Irena Čarman
Aleš Hvala

Recenzenti

Jože Arh, Breda Barbič-Žagar, Sonja Benčina-Crnić, Neda Benički-Švagelj, David Bratož, Nina Cimerman, Lea Cvitkovič-Maričič, Irena Čarman, Angela Čuk, Silvo Derganc, Jože Drinovec, Boris Dular, Dušan Dular, Branislav Filipović, Dragica Kozina, Aleš Gasparič, Dora Glažar, Jože Gnidovec, Mojca Golob, Mateja Gorjup, Marija Grčman, Urška Hočevar, Aleš Hvala, Marijan Ivanuša, Miha Japelj, Sandra Javernik, Peter Jerman, Sabina Jerman, Branko Kastelec, Ksenija Kikelj, Boštjan Korošec, Janko Kos, Pavel Kovač, Zdenka Kranjc-Gregorčič, Dragica Kozina, Dušan Krašovec, Andreja Kuhar, Peter Lenardič, Alenka Lukšič, Mateja Lukšič-Gorjanc, Alenka Malenšek, Jože Malenšek, Mateja Merlin, Marjo Merslavič, Mirjam Milharčič-Simčič, Marta Mohar, Damjan Možina, Milica Murn, Marko Oblak, Vesna Pahor, Diana Pavlič-Pokorny, Anica Pečavar, Tatjana Pečnik, Mitja Pelko, Andrej Petrišič, Irena Petrišič, Vladimir Petrovič, Robert Pišek, Andreja Plaper, Igor Plaper, Miroslav Pokorny, Vladimir Pokorny, Marjeta Potrč, Janja Požar, Simona Pregelj, Jože Primc, Aleša Punčuh Kolar, Ivan Radež, Anton Ignacij Recelj, Monika Reflak, Aleš Rotar, Lala Salom, Tomaž Sever, Igor Simonič, Peter Skubic, Gordan Sladič, Elizabeta Suhadolc, Mojca Štaudohar-Kozjan, Jaroslav Tihi, Robert Ucman, Sandra Urek-Blatnik, Anamarija Vajs, Boris Veselič, Nada Vinder, Miha Vrbinc, Stanislav Zalar, Pavla Zupančič, Silvo Zupančič, Slavko Zupančič, Damjana Zupančič-Božič, Rok Zupet.

Vsebina

32. Krkine nagrade / Krka Prizes

12. mednarodni simpozij / International Symposium
Zbornik povzetkov / Book of Abstracts

32. Krkine nagrade

32nd Krka Prizes

Uvodne besede

Letošnji simpozij in podelitev Krkinih nagrad potekata v znamenju odličnosti. To velja najprej za predavatelje, ki nas vsi po vrsti uvajajo v zapleteni svet narave in družbe.
Z enako mero odličnosti izraženo, samo na nekoliko drugačen način, pa lahko mirno označimo tudi delo nagrajencev. V njihovih nalogah se zrcali želja po ustvarjalnosti in doseganju ciljev. Posebej nas veseli, da je nabor obravnavanih tem enakomerno razporejen med različnimi področji, ki so sestavni del Krkinega življenja in razvoja.
Prepričani smo, da s tem Krkine nagrade izpolnjujejo svoje poslanstvo, ki sega prek meja zgolj uporabniškega gledanja na izobraževanje in raziskovanje.

Prepričani smo, da bomo to pot nadaljevali tudi v prihodnosti.

Opening Words

This year's symposium and Krka Prizes awarding are characterized by excellence. This applies first to the lecturers who all aim to introduce the complicated world of nature and society to us.
The same level of excellence, only expressed in a slightly different way, can be attributed also to the work of the award winners. Their assignments reflect a desire for creativity and attaining the objectives. We are particularly glad that the whole lot of discussed topics is uniformly distributed among various areas that constitute the life and development at Krka.

We are convinced that Krka Prizes fulfill their mission, which extends beyond the limits of viewing education and research just from the point of view of use.
We trust that we will continue in this direction also in the future.

Predsednik Sveta
Sklada Krkinih nagrad

President of
Krka Prizes Foundation

Dr. Aleš Rotar

Predsednik uprave in
generalni direktor Krke

Chief Executive and
President of the Management Board

Miloš Kovačič, mag. farm.

Krkini nagrajenci 2002 / Krka Prize Winners 2002

Št.	Nagrajenci	Status	Mentor, Somentor(ji)
1761	MATEJ BARIČ	študent farmacije	Odon PLANINŠEK
1762	ŽIGA BOLTA	podiplomski študent biotehnologije	Dea BARIČEVIČ
1763	KATARINA BORŠTAR	študentka farmacije	Samo KREFT
1764	DENIS CAR	študent visoke šole za upravljanje in poslovanje	Marjan BLAŽIČ
1765	MILAN CAR	dijak	Marinka KOVAČ
1766	SIMON ČADEŽ	študent ekonomije	Marko HOČEVAR
1767	ZDENKO ČASAR	doktorand, Francija	Dominique LORCY Alenka MAJCEN LE MARÉCHAL Miha JAPELJ Albert ROBERT
1768	NATAŠA DEBELJAK	doktorandka	Minoa RASSOULZADEGAN Damjana ROZMAN
1769	ANDREJ DOBROVOLJC	podiplomski študent informatike	Miran MIHELČIČ
1770	HOLGER DOBBEK	doktorand, Nemčija	Robert HUBER
1771	ANTONIJA DONKOVA-MARINOVA	študentka medicine, Bolgarija	Konstantin G. TCHERNEV
1772	EVGEN ERŽEN	doktorand	Božo PLESNIČAR
1773	KLEMENTINA FON TACER	podiplomska študentka medicine	Damjana ROZMAN
1774	JANEZ GODNOV	študent farmacije	Mitja KOS Vojko KMETEC
1775	MARKO GOLIČNIK	doktorand	Jure STOJAN
1776	MARIJA GRČMAN	doktorandka	Anton MEDEN Franc VREČER
1777	BORUT GREGORČIČ	študent strojništva	Iztok GOLOBIČ
1778	KLAVDIJA GRLICA	študentka biotehnologije	Ines MANDIČ-MULEC Aleš GASPARIČ
1779	IVA HAFNER	študentka kemije	Roman JERALA
1780	PETRA HUDLER	podiplomska študentka medicine	Stanislav REPŠE Radovan KOMEL
1781	DARIJA JAĆIMOVIĆ	študentka ekonomije	Miroslav GLAS
1782	ANDREJ JERINA	študent ekonomije	Marko BOŠNJAK
1783	JANEZ JERMAN	študent farmacije	Odon PLANINŠEK
1784	TATJANA JOVIĆ	študentka družbenih ved	Ivan SVETLIK Alenka PUČKO
1785	MARKO JUG	študent elektrotehnike	Stanislav KOVAČIČ Brane DEŽMAN
1786	BRIGITA KALČIČ	študentka visoke šole za zdravstvo	Eva BATAGELJ Irena ČARMAN Tatjana HARLANDER
1787	JURE KAPETAN	študent ekonomije	Dušan MRAMOR
1788	MARIJA AJA KOCUVAN	študentka biologije	Nina GUNDE-CIMERMAN
1789	KATJA KOČEVAR	študentka ekonomije	Mladen KUČIŠ Vesna ŽABKAR
1790	PIOTR KOŁSUT	doktorand, Poljska	Zbigniew RELIGA
1791	JURE KORENT	študent ekonomije	Boštjan JAZBEC
1792	JASNA KOS	študentka kemije	Lucija ZUPANČIČ-KRALJ Jolanda KUNEJ
1793	MATEJA KOŠIČEK	študentka organizacijskih ved	Vladislav RAJKOVIČ Nevenka BANOVEC
1794	ANITA KOVAČ-KRALJ	doktorandka	Peter GLAVIČ Zdravko KRAVANJA
1795	ENEJ KUŠČER	študent biotehnologije	Radovan KOMEL
1796	ALF LAMPRECHT	doktorand, Nemčija	Claus-Michael LEHR
1797	POLONCA LEGAN	dijakinja	Marinka KOVAČ
1798	TANJA LESKOŠEK	študentka farmacije	Robert PIŠEK Franc VREČER
1799	MATEJ LUŠTEK	študent veterine	Valentin SKUBIC

1800	MONIKA MEDVEŠEK	študentka farmacije	Stane SRČIČ Natalija ZAJC
1801	GRIŠA MOČNIK	doktorand	Mladen FRANKO Dragan D. MIHAILOVIĆ
1802	NINA MURKO	dijakinja	Ksenija KIKELJ
1803	KRISTINA NADRAH	študentka farmacije	Marija SOLLNER DOLENC
1804	KLEMEN NAVERŠNIK	študent farmacije	Slavko PEČAR
1805	NATALIJA PERME	študentka kemije	Nataša BUKOVEC Sabina GRABNER
1806	JANEZ PERŠ	študent elektrotehnike	Stanislav KOVAČIČ Brane DEŽMAN
1807	KATJA PLAPER	dijakinja	Andreja PLAPER Natalija PETAKOVIČ
1808	BLANKA PREMROV	študentka biologije	Vladka ČURIN-ŠERBEC Vesna GALVANI
1809	MAJA PRESKAR	študentka farmacije	Franc VREČER
1810	TANJA PUCELJ	študentka farmacije	Jana LUKAČ-BAJALO Samo KREFT
1811	TADEJA RAJER	študentka farmacije	Janja MARC
1812	UROŠ RAJKOVIČ	študent organizacijskih ved	Jože M. ZUPANČIČ
1813	MIJA RANGUS	dijakinja	Ksenija KIKELJ
1814	ALEKSANDAR RAŠKOVIĆ	podiplomski študent medicine, ZR Jugoslavija	Momir MIKOV
1815	SIMON REČNIK	doktorand	Jurij SVETE Branko STANOVNIK
1816	TINA ROBLEK	študentka farmacije	Slavko PEČAR
1817	ANA SEČNIK	podiplomska študentka, Belgija	Robert HINE
1818	MATEJ SELIŠKAR	študent farmacije	Borut ŠTRUKELJ Damjana ROZMAN
1819	BARBARA SENEKOVIČ	študentka višje gostinske šole	Miran ČURIN
1820	TANJA SITAR	študentka farmacije	Vojko KMETEC Robert ROŠKAR
1821	VLADIMIR V. SIZONOV	podiplomski študent medicine, Rusija	Michael I. KOGAN
1822	ŠPELA SMRKOLJ	podiplomska študentka medicine	Stelio RAKAR
1823	MOJCA STRAŽIŠAR	študentka biotehnologije	Aleš GASPARIČ
1824	ANITA ŠKOF	podiplomska študentka farmacije	Aleš KRBAVČIČ Mario POLJAK
1825	BOŠTJAN ŠTAMCAR	študent zootehnike	Antonija HOLCMAN
1826	PETER ŠTUKELJ	študent kemijske tehnologije	Valentin KOLOINI Ivan RADEŽ
1827	DARKO ŠUŠTARŠIČ	podiplomski študent ekonomije	Zvezdana BAJC
1828	JASNA ŠUŠTARŠIČ	študentka medicine	Metka V. BUDIHNA Gorazd DREVENŠEK
1829	IVAN TOPLAK	podiplomski študent veterine	Jože GROM Darja BARLIČ-MAGANJA
1830	REBEKA TOPORIŠIČ	študentka kemije	Boris PIHLAR
1831	BARBARA TURK	dijakinja	Ksenija KIKELJ
1832	KSENIJA TURK	dijakinja	Andreja PLAPER Natalija PETAKOVIČ
1833	MONIKA TURK	dijakinja	Marinka KOVAČ
1834	MARTINA VODOPIVEC	doktorandka	Marin BEROVIČ Aleš PODGORNIK
1835	VALENTINA VRBINC	študentka fizioterapije	Miroljub JAKOVLJEVIĆ Marijana KUHAR
1836	GORDANA ZDUNIĆ	študentka farmacije, ZR Jugoslavija	Nenad UGREŠIĆ
1837	LUCIJA ZUPANČIČ	študentka kemijskega inženirstva	Janvit GOLOB

32. Krkine nagrade
32nd Krka Prizes

12. mednarodni simpozij
12th International Symposium
Zbornik povzetkov
Book of Abstracts

12. mednarodni simpozij ob 32. Krkinih nagradah

Krkine nagrade, ki pomenijo posebno spodbudo za mlade in ustvarjalne ljudi, so posvečene predvsem znanju in spoznavanju pomembnih znanstvenih, razvojnih in raziskovalnih dosežkov, ki so ključnega pomena za smotrni razvoj sodobne farmacevtske industrije.
Svečane podelitve Krkinih nagrad so postale posebna priložnost, da se mladi študentje in dijaki pri nas v Krki, v Novem mestu srečujejo in spoznavajo s številnimi profesorji in učitelji, raziskovalci in strokovnjaki z univerz, iz 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 pred dvanajstimi leti pričeli povezovati Krkine nagrade z organizacijo mednarodnih simpozijev. V našo sredino radi vabimo vrhunske znanstvenike, strokovnjake in raziskovalce s celega sveta. Na naših simpozijih obravnavamo moderne in raznolike znanstvene in razvojne tematike, ki so zelo navezane na našo farmacevtsko-kemijsko industrijo. V teh dvanajstih letih smo v Krko kot plenarne predavatelje povabili že nad 80 uveljavljenih vrhunskih univerzitetnih profesorjev, akademikov in strokovnjakov iz 20 držav Evrope in ZDA in Slovenije.
Na naših mednarodnih simpozijih predstavljamo tudi raziskovalne dosežke Krkinih nagrajencev. S svojimi predavanji sodelujejo Krkini nagrajenci doktorji znanosti, drugi Krkini nagrajenci pa predstavljajo svoje dosežke v obliki posterjev. Letos smo v Krko povabili priznane akademike, profesorje in strokovnjake iz sedmih držav, iz Nemčije, Rusije, Poljske, Madžarske, Bolgarije, Jugoslavije in Slovenije. Posebej pa nas veseli, da je naše povabilo za uvodno plenarno predavanje sprejel nobelovec prof. dr. Robert Huber, ki ga danes uvrščamo med najpomembnejše in najuspešnejše biokemike in kemike na svetu.

Na 12. mednarodnem simpoziju, ki ga bo slavnostno odprl predsednik Slovenske akademije znanosti in umetnosti, **akademik prof. dr. Boštjan Žekš,**

bodo sodelovali naslednji vabljeni plenarni predavatelji:

Nobelovec akademik prof. dr. Robert Huber iz Nemčije
Prof. dr. Robert Huber se je v svetu vidno uveljavil pri biokemijskih raziskavah proteolitskih encimov in njihovih biogenih zaviralcev. Temeljito je proučeval zaviralce cisteinskih proteinaz in strukture izbranih beljakovin. Središčna vloga njegovih raziskav pa je usmerjena na razvoj sodobnih metod proteinske kristalografije, njenih izračunov in ustrezne računalniške grafike. Leta 1988 je prejel Nobelovo nagrado za kemijo (skupaj z J. Deisenhoferjem in H. Michelom) za dosežke pri določitvi prostorske strukture fotosintetičnega reakcijskega centra.
Profesor Huber je direktor oddelka za strukturne raziskave v Inštitutu Max Planck in pomemben član številnih akademij znanosti in društev za biokemijo, biofiziko in kemijo po vsem svetu. Posebej pa moramo omeniti njegovo dolgoletno sodelovanje z IJS in s slovenskimi znanstveniki in raziskovalci, ki so se v Inštitutu Max Planck znanstveno usposabljali in izvajali svoja doktorska dela.

Prof. dr. András Inotai iz Madžarske
Prof. dr. András Inotai se uvršča s svojimi številnimi publikacijami in knjigami med vrhunske strokovnjake v svetu na področju ekonomskih znanosti. Je generalni direktor Inštituta za svetovno ekonomijo pri Madžarski akademiji znanosti in umetnosti. Profesor Inotai se uveljavlja s predavanji s področja ekonomije na univerzah v številnih državah Evrope in Amerike. Nedavno je tudi vodil strateški odbor za integracijo v Evropsko Unijo. Je tudi član Foruma Bled, kjer nas seznanja s dosežki in trendi na področju svetovne ekonomije.

Prof. dr. Zbigniew Religa iz Poljske
Prof. dr. Zbigniew Religa je vrhunski kardiolog na Poljskem in je tudi vidno uveljavljen v svetu. Je generalni direktor Nacionalnega inštituta za kardiologijo v Varšavi. Strokovno se je usposabljal v ZDA na področjih vaskularne in srčne kirurgije. Profesor Religa je tudi izumitelj novih bioloških srčnih zaklopk in uveljavlja najnovejše svetovne in svoje dosežke iz področja srčne kirurgije pri svoji izredno pestri in zahtevni medicinski praksi.
Profesor je danes senator Republike Poljske in častni doktor na štirih univerzah Poljske in Ukrajine.

Prof. dr. Michael Iosipovch Kogan iz Rusije
Profesor Kogan spada med danes najpomembnejše urologe v Rusiji. Je direktor Katedre za urologijo Rostovske medicinske fakultete in predsednik združenja urologov Dona in član ruskih in evropskih združenj urologov. Profesor Kogan je ob svoji zelo bogati medicinski praksi objavil tudi vrsto znanstvenih in strokovnih knjig in revij ter prijavil 20 izumov iz področij onkologije in andrologije. Spada med pomembne univerzitetne profesorje na Univerzi v Rostovu na Donu.

Prof. dr. Claus-Michael Lehr iz Nemčije
Profesor Lehr je vodja oddelka za biofarmacevtiko in farmacevtsko tehnologijo na Univerzi Saarland v Saarbrücknu. V letu 1998 je bil soustanovitelj firme ACROSS BARRIERS GmbH, ki nudi validirane celične kulture in analitsko podporo pri načrtovanju zdravil.
Profesor Lehr posveča svoj znanstveni in raziskovalni interes kontroliranemu transportu makromolekularnih zdravil, kot so peptidi, proteini in genski vektorji. V svojih laboratorijih razvija bioadhezijske tehnologije, koloidne nosilce zdravil, nevirusne genske transportne sisteme in tkivni inženiring. Profesor Lehr je član številnih tujih in nemških združenj, je urednik European Journal of Pharmaceutics and Biopharmaceutics in tudi član uredniških odborov pri sorodnih tujih revijah. Njegova raziskovalna skupina je lani prejela visoki nagradi Phoenix in Scheele pri nemškem farmacevtskem društvu.

Prof. dr. Konstantin Georgiev Tchernev iz Bolgarije
Profesor Tchernev je predstojnik Oddelka za interno medicino na Kliniki za gastroenterologijo na Aleksandrovi bolnišnični univerzi v Sofiji. Svoje uspešno pedagoško, strokovno in raziskovalno delo usmerja na področjih kroničnega hepatitisa, instrumentalne diagnostike, akutnega in kroničnega pankreatitisa in na drugih področjih. Je član evropskih in bolgarskih združenj in društev za interno medicino. Strokovno in znanstveno se je izpopolnjeval v Nemčiji in bil večkrat tudi gostujoči profesor v tujini. Profesor Tchernev spada med vodilne interniste, gastroenterologe v Bolgariji.

Prof. dr. Momir Mikov iz Jugoslavije
Profesor Mikov je profesor farmakologije in toksikologije na Univerzi v Novem Sadu. Prednostno poučuje, vodi in usmerja študij in raziskave na področjih metabolizma zdravil *in vivo* in *in vitro*, razvija analitske metode za študije metabolizma in načrtuje sodobne klinične raziskave. Je avtor številnih publikacij, knjig in izbranih patentov. Znanstveno in strokovno se je izpopolnjeval v Angliji in na Hrvaškem. Je član številnih jugoslovanskih in svetovnih združenj in društev. Profesor Mikov je tudi osnoval regionalni center za zdravila in strupe.

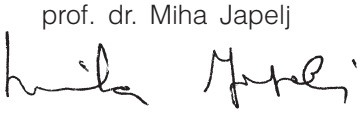
Prof. dr. Borut Štrukelj iz Slovenije
Profesor Štrukelj je prodekan na Fakulteti za farmacijo v Ljubljani, kjer vodi skupino za farmacevtsko biotehnologijo, in vodja programske skupine za rastlinsko molekularno biologijo na Inštitutu Jožef Stefan. Podoktorsko se je izpopolnjeval na Inštitutu za rastlinsko molekularno biologijo (CPRO-DLO, PRI Wageningen), v Wageningenu na Nizozemskem. Je soavtor izbranih svetovnih patentnih prijav in patenta na področju transgenih rastlin in avtor številnih znanstvenih in strokovnih člankov v tujih in domačih revijah. Za raziskovalne dosežke je že prejel nagrado Kidričevega sklada. Profesor Štrukelj je član svetovnih, evropskih in slovenskih združenj s področja farmacije in molekularne biologije. Od leta 1996 deluje kot izvedenec v Evropski farmakopeji (Strassbourg) v skupini za farmacevtske biomolekule.

Svoja doktorska dela bodo predstavili tudi Krkini nagrajenci, novi doktorji znanosti:
Holger Dobbek iz Nemčije, Alf Lamprecht iz Nemčije, Piotr Kołsut iz Poljske, Zdenko Časar iz Francije, in Griša Močnik, Evgen Eržen, Simon Rečnik, Anita Kovač Kralj, Marija Grčman, Martina Vodopivec, Nataša Debeljak in Marko Goličnik, vsi iz Slovenije.

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.

Profesorju Huberju, vsem vabljenim plenarnim predavateljem in vsem Krkinim nagrajencem se prisrčno zahvaljujemo. V našo Krko ste prinesli spet nova znanja in spoznanja in nove, vrhunske znanstvene ter raziskovalne dosežke. K nam prinašate nove raziskovalne in razvojne izzive ter novo sinergijo našega nadaljnjega sodelovanja.

Predsednik odbora
12. mednarodnega simpozija
prof. dr. Miha Japelj



12th International Symposium and 32nd Krka Prizes

Krka Prizes, which represent a special stimulation for young and creative people, are dedicated mainly to knowledge and learning about important scientific, developmental and research achievements which are of vital importance for proper development of modern pharmaceutical industry.
Krka Prize awarding ceremonies have become a special opportunity for young secondary school and university students to meet at Krka in Novo mesto and become acquainted with many professors and teachers, researchers and experts from universities, numerous domestic and foreign institutes and from the pharmaceutical industry. These meetings give rise to new acquaintances and friendships, new plans and co-operations. For this reason, we started to interlink Krka Prizes with the organization of international symposiums 12 years ago. We like to invite prominent scientists, experts and researchers from all over the world. At our symposiums, modern and diverse scientific and developmental topics that are closely related to our pharmaceutical-chemical industry are dealt with. In the past 12 years, we have invited to Krka over 80 renowned and prominent university professors, academicians and experts from 20 countries from Europe, the USA and Slovenia as plenary lecturers.
At our international symposiums, we also present the research results of Krka Prize winners. Doctors of Science participate as lecturers, other prize winners present their research results in the form of posters. This year we have invited to Krka renowned academicians, professors and experts from seven countries: Germany, Russia, Poland, Hungary, Bulgaria, Yugoslavia and Slovenia. It is a great pleasure for us that Prof. Dr. Robert Huber, a Nobel Prize winner and one of the most important and successful biochemists and chemists in the world, has accepted our invitation to give the opening plenary lecture.

At 12th International Symposium, which will be officially opened by the President of the Slovenian Academy of Sciences and Arts, **Acad. Prof. Dr. Boštjan Žekš,**

the following plenary lecturers will take part:

Nobel Prize winner, Acad. Prof. Dr. Robert Huber from Germany
Prof. Dr. Robert Huber has won recognition in the world for the biochemical researches of proteolytic enzymes and their biogenic inhibitors. He has thoroughly studied cysteine proteinase inhibitors and the structures of selected proteins. The central role of his researches is directed towards the development of up-to-date methods of protein crystallography, its calculations and adequate computer graphics. In 1988 he was awarded the Nobel Prize in chemistry (together with J. Deisenhofer and H. Michel) for the achievements in the determination of three-dimensional structure of a photosynthetic reaction centre.
Professor Huber is the Director of the Department for Structural Researches at the Max-Planck-Institut and an important member of numerous Academies of Sciences and societies for biochemistry, biophysics and chemistry worldwide. Special mention should be made of his long-standing co-operation with IJS and the Slovenian scientists and researchers whose scientific training and implementation of doctoral dissertations took place at the Max-Planck Institut.

Prof. Dr. András Inotai from Hungary
With his numerous publications and books, Prof. Dr. András Inotai ranks among the world's top experts in the field of economic sciences. He is the General Manager of the Institute for World Economicof the Hungarian Academy of Sciences. Prof. Inotai is known for his lectures from the field of economy at universities in many European and American countries. He was the heas of the Strategic Task Force on integration into European Union.
Finally, he is also a member of the Forum in Bled, where he keeps us informed about the achievements and trends in the field of world economy.

Prof. Dr. Zbigniew Religa from Poland
Prof. Dr. Zbigniew Religa is one of the leading cardiologists in Poland and is also recognized worldwide. He is the General Manager of the National Institute for Cardiology in Warsaw. His further professional training in the field of vascular and cardiac surgery took place in the USA. Professor Religa has also invented new biological cardiac valves and introduces the latest world's and his own achievements from the field of cardiac surgery into his very rich and demanding medical practice.
Today Professor Religa is a senator of the Republic of Poland and an honorary doctor at four universities in Poland and Ukraine.

Prof. Dr. Michael Iosipovch Kogan from Russia
Professor Kogan ranks among the most important urologists in Russia. He is the Director of the Chair in Urology at the Rostov State Medical University, president of the Urologists' Association of the Don and a member of the Russian and European urologists' associations. In addition to his very rich medical practice, Professor Kogan has published many scientific and technical books and magazines and patented 20 inventions from the field of oncology and andrology. He is one of the most important university professors at the University in Rostov-on-Don.

Prof. Dr. Claus-Michael Lehr from Germany
Professor Lehr is the Head of the Department for Biopharmaceutics and Pharmaceutical Technology at the University Saarland in Saarbrücken. In 1998 he was a co-founder of the company ACROSS BARRIERS GmbH, which provides validated cell cultures and analytical support in planning medicinal products.
Professor Lehr dedicates his scientific and research interest to controlled transport of macromolecular agents, such as peptides, proteins, and gene vectors. In his laboratories, he develops bioadhesive technologies, colloidal agent carriers, non-virus gene transport systems and tissue engineering. Professor Lehr is a member of numerous foreign and German associations, the editor of European Journal of Pharmaceutics and Biopharmaceutics and also a member of editorial boards with related foreign journals. Last year his research team was awarded the outstanding prizes Phoenix and Scheele by the German Pharmaceutical Society.

Prof. Dr. Konstantin Georgiev Tchernev from Bulgaria
Professor Tchernev is the Head of the Department for Internal Medicine at the Clinic for Gastroenterology at the Medical Faculty in Sofia. His successful teaching, professional and research activities are focused on the field of chronic hepatitis, instrumental diagnostics, acute and chronic pancreatitis and also other fields. He is a member of European and Bulgarian associations and societies for internal medicine. He went to Germany for further professional and scientific training and he has often been a visiting professor abroad. Professor Tchernev is one of the leading internal specialists, gastroenterologists in Bulgaria.

Prof. Dr. Momir Mikov from Yugoslavia
Professor Mikov is a professor of pharmacology and toxicology at the University of Novi Sad. His priority is teaching, leading and directing studies and researches in the field of drug metabolism in vivo and in vitro, developing analytical methods for the studies of metabolism and designing up-to-date clinical trials. He is the author of numerous publications, books and selected patents. He continued his scientific and professional training in England and Croatia. He is a member of many Yugoslav and world associations and societies. Professor Mikov also established the regional center for medicinal products and poisons.

Prof. Dr. Borut Štrukelj from Slovenia
Prof. Dr. Borut Štrukelj is the Deputy Dean at the Faculty of Pharmacy, Ljubljana where he leads a group for pharmaceutical biotechnology. He is the head of the plant molecular biology group at the Jožef Stefan Institute. By using post-doc EMBO award, he worked at the CPRO-DLO, PRI Institute for plant molecular biology in Wageningen, Netherlands. He is a co-author of selected world patent applications and the patent in the field of transgenic plants. He is also the author of numerous scientific and technical articles in foreign and domestic journals. For his research achievements, he has already been awarded the Kidrič award. Professor Štrukelj is a member of international, European and Slovenian associations from the field of pharmacy and molecular biology. Since 1996 he has worked as an expert in the European Pharmacopoea (Strassbourg), in the group for pharmaceutical biomolecules.

The following **Krka Prize winners** who are also new Doctors of Science will present their doctoral dissertations:

Holger Dobbek from Germany, Alf Lamprecht from Germany, Piotr Kołsut from Poland, Zdenko Časar from France, and Griša Močnik, Evgen Eržen, Simon Rečnik, Anita Kovač Kralj, Marija Grčman, Martina Vodopivec, Nataša Debeljak and Marko Goličnik, all from Slovenia.

Other Krka Prize winners will present their research achievements obtained in their undergraduate and master degree studies in the Poster section. The youngest Krka Prize winners, i.e. secondary school students from the Dolenjska region, will also present their research assignments.

We wish to express our warmest thanks to Professor Huber, all the invited plenary lecturers and all Krka Prize winners for enriching us once again with new aspects of knowledge, new findings and new and top-level scientific and research achievements. You bring us new research and development challenges and a new synergy of our further co-operation.

Chairman of the Scientific Board
of 12th International Symposium
Prof. Dr. Miha Japelj

32nd Krka Prizes

Programme of the 12th International Symposium

Novo mesto, 24th of October 2002

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

9.10 - 9.20 **Opening of the Symposium – Boštjan Žekš**

1 Plenary Lectures - Part I.

Chairman: Miha Japelj

9.20 - 10.05 Molecular Machines for Protein Degradation 29
Robert Huber
Max-Planck-Institut für Biochemie, Martinsried, Germany

10.05 - 10.35 On the Eve of the Enlargement of the European Union 30
András Inotai
Institute for World Economics of the Hungarian Academy of Sciences, Budapest, Hungary

10.35 - 10.45 **Break**

10.45 - 11.15 Present Status of Clinical Application of Mechanical Heart Assist Devices 31
Zbigniew Religa
National Institute of Cardiology, Warsaw, Poland

11.15 - 11.45 Ciprofloxacine for Antimicrobial Prophylaxis of Urinary Tract Infections in Patients Undergoing Transurethral Resection of Prostate 32
Michael I. Kogan
Rostov State Medical University, Rostov-on-Don, Russia

11.45 - 12.05 **Coffee Break**

Plenary Lectures - Part II.

Chairman: Miha Japelj

12.05 - 12.35 Pharmaceutical Nano-Biotechnology: Perspectives for Advanced Drug Delivery 33
Claus-Michael Lehr
Saarland University, Saarbrücken, Germany

12.35 - 13.05 The Impact of Modern Biotechnology on the Pharmaceutical, Medical and Environmental Development 35
Borut Štrukelj
Faculty of Pharmacy, University of Ljubljana, Slovenia

13.05 - 13.35 *Helicobacter pylori* Infection – Diagnosis and Treatment 36
Konstantin G. Tchernev
Medical Faculty, Sofia, Bulgaria

13.35 – 14.05 Metabolism of Drugs and Other Xenobiotics by Gut Microflora 37
Momir Mikov
Medical Faculty, Novi Sad, FR Yugoslavia

14.05 – 15.00 **Lunch Break**

2 Krka Prize Winners' Presentations

Chairman: Aleš Rotar

15.00 - 15.15 Aerobic and Anaerobic Life on Carbon Monoxide Oxidation 41
Holger Dobbek
Max-Planck-Institut für Biochemie, Martinsried, Germany

15.15 - 15.30 Nanoparticle Targeting as a Therapeutic Approach in Inflammatory Bowel Disease 42
Alf Lamprecht
Saarland University, Saarbrücken, Germany

15.30 - 15.45 Gene Therapy of Coronary Artery Disease in Patients Undergoing Surgical Incomplete Myocardial Revascularization 43
Piotr Kołsut
National Institute of Cardiology, Warsaw, Poland

15.45 - 16.00 Diselenadiazafulvalenes: Novel Electron Rich Olefins Precursors of Organic Materials 44
Zdenko Časar
Université de Rennes 1, Rennes, France

16.00 - 16.15 Photothermal Methods for Investigation of Highly Absorbing and Scattering Samples 45
Griša Močnik
Jožef Stefan Institute, Ljubljana, Slovenia

16.15 - 16.30 *T*he Reactive Intermediates in the Ozonation of Saturated Hydrocarbons 46
Evgen Eržen
Faculty of Chemistry and Chemical Technology, University of Ljubljana, Slovenia

16.30 - 16.45 Syntheses and Transformations of Stable Heteroaryl-Diazonium Salts 47
Simon Rečnik
Faculty of Chemistry and Chemical Technology, University of Ljubljana, Slovenia

16.45 - 17.00 Energy – Mass Retrofit of Complex Continuous Processes 48
Anita Kovač Kralj
Faculty of Chemistry and Chemical Technology, University of Maribor, Slovenia

17.00 - 17.15 Characterization of Polymorphic Forms of Doxazosin Mesylate and Its Amorphous Phase 49
Marija Grčman
Faculty of Chemistry and Chemical Technology, University of Ljubljana, Slovenia

17.15 - 17.30 Process Separation and Determination of Bioprocess Metabolites on Monolithic Support 50
Martina Vodopivec
National Institute of Chemistry, Ljubljana, Slovenia

17.30 - 17.45 Mouse as a Model Organism to Study the Role of the *Cyp51* Gene in Spermatogenesis 51
Nataša Debeljak
Faculty of Medicine, University of Ljubljana, Slovenia

17.45 - 18.00 Mechanism of Cooperative Phenomena by Cholinesterases 53
Marko Goličnik
Faculty of Medicine, University of Ljubljana, Slovenia

18.00 - 18.15 **Discussion and Final Remarks**

3 Krka Prize Winners' Poster Session

will be displayed during the duration of the entire event
Chairman: Stanislav Zalar

Determination of Surface Properties of Lactose with Inverse Gas Chromatography 57
Matej Barič, Janez Jerman
Faculty of Pharmacy, University of Ljubljana, Slovenia

Optimisation of Ursolic Acid Production from Sage *(Salvia Officinalis* L.*)* in Bioreactor 58
Žiga Bolta
Biotechnical Faculty, University of Ljubljana, Slovenia

Development of Isolation Method for Thrombin Inhibitors from *Stereum hirsutum* 59
Katarina Borštar
Faculty of Pharmacy, University of Ljubljana, Slovenia

Marketing Communication through Media 60
Denis Car
School of Business and Management, Novo mesto, Slovenia

The Role of Accounting Information in Attainment of Strategic Goals of Slovenian Companies 61
Simon Čadež
Faculty of Economics, University of Ljubljana, Slovenia

The Implementation of a Process-Oriented Organization through Object Oriented Modelling and »Rapid Re« Methodology 62
Andrej Dobrovoljc
Faculty of Computer and Information Science, University of Ljubljana, Slovenia

Norfloxacine – Primary and Secondary Prophylaxis of Spontaneous Bacterial Peritonitis in Patients with Cirrhosis and Ascites 63
Antonia Donkova - Marinova
Medical Faculty, Sofia, Bulgaria

Tissue-Specific Regulation of Cholesterogenic Genes Expression 64
Klementina Fon Tacer
Faculty of Medicine, University of Ljubljana, Slovenia

32.
Krkine
nagrade
32nd
Krka
Prizes

1 Plenary Lectures

Molecular Machines for Protein Degradation

R. Huber
Max-Planck-Institut für Biochemie, Martinsried, Germany

Within cells or subcellular compartments misfolded and/or short-lived regulatory proteins are degraded by protease machines, cage-forming multi-subunit assemblages. Their proteolytic active sites are sequestered within the particles and located on the inner walls. Access of protein substrates is regulated by protein subcomplexes or protein domains, which may assist in substrate unfolding dependent of ATP. Four protease machines will be described displaying different subunit structures, oligomeric states, enzymatic mechanisms, and regulatory properties.

Proteasome

Groll, M., Ditzel, L., Löwe, J., Stock, D., Bochtler, M., Bartunik, H. D. and Huber, R. Structure of 20S proteasome from yeast at 2.4 A resolution. *Nature* **1997**, *386*, 463-471.
Groll, M., Heinemeyer, W., Jäger, S., Ullrich, T., Bochtler, M., Wolf, D. H. and Huber, R. The catalytic sites of 20S proteasomes and their role in subunit maturation: A mutational and crystallographic study. *Proc. Natl. Acad. Sci. USA* **1999**, *96*, 10976-10983.
Groll, M., Bajorek, M., Köhler, A., Moroder, L., Rubin, D. M., Huber, R., Glickman, M. H. and Finley, D. A gated channel into the proteasome core particle. *Nature Struct. Biol.* **2000**, *7*, 1062-1067.

HslV/HslU

Bochtler, M., Hartmann, C., Song, H. K., Bourenkov, G., Bartunik, H. and Huber, R. The structure of HslU and the ATP-dependent protease HslU-HslV. *Nature* **2000**, *403*, 800-805.
Song, H. K., Hartmann, C., Ramachandran, R., Bochtler, M., Behrendt, R., Moroder, L. and Huber, R. Mutational studies on HslU and its docking mode with HslV. *Proc. Natl. Acad. Sci. USA* **2000**, *97*, 14103-14108.
Ramachandran, R., Hartmann, C., Song, H. J., Huber, R. and Bochtler, M. Functional interactions of HslV(ClpQ) with the ATPase HslU(ClpY). *Proc. Natl. Acad. Sci. USA* **2002**, *99*, 7396-7401.

Tricorn

Brandstetter, H., Kim, J. S., Groll, M. and Huber, R.Crystal structure of the tricorn protease reveals a protein disassembly line. *Nature* **2001**, *414*, 466-470.
Kim, J. S., Groll, M., Musiol, H. J., Behrendt, R., Moroder, L., Huber, R. and Brandstetter H. Navigation inside a protease: substrate selection and product exit in the tricorn protease from Thermoplasma acidophilum. **2002** *(submitted)*.
Goettig, P., Groll, M., Kim, J. S., Huber, R. and Brandstetter, H. Structures of tricorn interacting aminopeptidase F1 with different ligands explain its catalytic mechanism. **2002** *(submitted)*.

DegP(HtrA)

Krojer, T., Garrido-Franco, M., Huber, R., Ehrmann, M., and Clausen, T. Crystal structure of DegP (HtrA) reveals a new protease-chaperone machine. *Nature* **2002**, *416*, 455-459.

On the Eve of the Enlargement of the European Union

A. Inotai
Institute for World Economics of the Hungarian Academy of Sciences, Budapest, Hungary

It is likely that the EU will enlarge in 2004. From various aspects, it will be a new kind of enlargement. First, it takes place in the process of globalization, which challenges companies, national economies and regional integrations alike. Second, most probably it will include up to ten countries on rather different levels of economic and social development, which certainly will affect their adjustment and absorption capacity once they are in the Union. Third, at least in GDP per capita terms, all countries are poorer than the EU average and will be entitled to EU funds.
At the same time, the EU`s internal cohesion will also be challenged, since the politically motivated big bang enlargement, unlike the Mediterranean one, seems to have insufficient economic resources to compensate for the consequences of a political decision. Conflicts of basic priorities of the integration as well as financial issues may create difficult situations both between the present and future member countries and among the present ones alike.
However, the enlargement offers a number of new opportunities: larger markets, better investment framework, additional R+D capacities, stronger growth and structural modernization. In addition, the coming enlargement will include geographically core continental countries which are to a large extent transit economies. Therefore, the structural funds have to consider the regional implications of infrastructure and environmental investments after 2007.
As a result of the big bang enlargement, the European Union's Eastern borders will be reached, while the southern and southeastern borders remain soft borders with countries, which sooner or later are likely to join the Union. Therefore, the EU has to develop a clear strategy towards the not-yet member countries of the region and remain open for further enlargement.
The enlargement will substantially change the domestic economic policies of the new members. Major impacts can be expected in the volume, structure and geographic orientation of their trade relations, with above average growth among the new member countries. Also the attitude of foreign investors may be changing. Implications will be strongest in those sectors, which are in the forefront of technological development. The pharmaceutical industry certainly belongs to this group. Therefore the lecture will also address some key issues of this industry.

Present Status of Clinical Application of Mechanical Heart Assist Devices

Z. Religa
National Institute of Cardiology, Warsaw, Poland

Currently there are many types of mechanical devices that are used to support failing heart. For many years they were clinically applied as a bridge to recovery or as a bridge to heart transplantation. Recently some of the newly developed devices become an alternative to heart transplantation and they are used as a permanent implant.
The most common indication for mechanical support is postcardiotomy heart failure and usually in such cases intraaortic balloon is the first choice. Unfortunately it does not provide the level of support that is needed in cases of severe cardiogenic shock. In these situations surgeon can use centrifugal or pneumatic pumps for short-term support.
Another group of patients who can benefit from short-term mechanical support are patients with fulminant myocarditis.
When the long-term support is needed, like in patients waiting for heart transplantation, partially implantable electromechanical devices seems to be the best solution. Very promising are novel axial flow assist devices that might be used for long term and even permanent implantation. In patients with severe heart failure but with contraindications to heart transplant implantable total artificial heart as permanent solution was used in year 2001.
In this presentation advantages and disadvantages of different mechanical heart assist devices will be presented, including also devices produced in Poland.

Ciprofloxacine for Antimicrobial Prophylaxis of Urinary Tract Infections in Patients Undergoing Transurethral Resection of Prostate - Open Multicenter Prospective Randomized Study in Russia (Intermediate Analysis)

M. I. Kogan
Rostov State Medical University, Rostov-on-Don, Russia

Introduction and objective: TURP is a "gold" standard of the surgical treatment of BPH with a high frequency rate of postoperative urinary tract infection (6-22%). Preoperational prophylaxis is one of the ways to prevent bacterial complications, particularly in patients with risk factors. As an antibiotic various agents are used, however greatest application have received fluorochinolones, and among them - ciprofloxacine. At present different patterns of antibiotic prophylaxis are being studied. The main objective of our study is to compare efficacy and safety of two patterns of ciprofloxacine introduction.
Materials and methods: 153 patients divided to three equal groups should be examined: I - 500 mg ciprofloxacine per os 90-120 min. before TURP, II - 400 mg ciprofloxacine intravenously 30-60 min. before TURP, III - antibiotic prophylaxis before TURP is not conducted.
Selection criteria for patients to be examined conformed to the objective. Clinical and bacteriological urine examinations with determination of microbial number, causative agent, and ciprofloxacine susceptibility were carried out 1 day before TURP and also in 1, 7, and 14 days following TURP.
Results: 62 patients were examined: Group I - 19, II - 21 and III - 22 patients. Mean age was 65,5 ± 8,8 years. There are no differences in background pathology and duration of surgery between the groups. Rate of UTI onset following TURP:

Period of study followingTURP	Group 1		Group 2		Group 3	
	N	%	N	%	N	%
1st day	-	-	2	9,5	2	10,5
7th day	1	5,3	4	19,0	4	21,1
14th day	1	5,3	6	28,6	5	26,3

Necessity of antibiotics administration following the surgery: Group I - 17,65%, Group II - 25,0%, Group III - 60,0% cases.
Conclusion: Ciprofloxacine per os before TURP results in reliable ($p<0,001$) decrease of UTI frequency rate as compared to groups II and III of patients, and also in reliable decrease of need in postoperative antibiotic administration. Tendency to reduction of the hospital treatment period was noted for Group I patients.

Pharmaceutical Nano-biotechnology: Perspectives for Advanced Drug Delivery

C.-M. Lehr
Saarland University, Saarbrücken, Germany

Pharmaceutical nano-biotechnology has an enormous potential to improve the delivery of drugs across biological barriers, both for local as well as for systemic application. This holds in particular for macromolecular biopharmaceuticals (peptides, proteins, antisense agents, gene vectors), but also for conventional small organic molecules.
In our laboratories, we are exploring the potential of nanoparticulate carrier systems for controlled drug and gene delivery at epithelial cells. This may lead to novel drug delivery systems for oral application, but the principle should also be transferable to other epithelial barriers/sites of application, such as e.g. the respiratory tract, the skin or blood vessel endothelia.
Our approach comprises the strategic development of:

- New *in vitro* models of biological barriers based on human epithelial cells and tissues (1)
- New platform technologies for the preparation of nanometer-sized drug carriers;
- Surface modification with bio-specific ligands to control cellular binding, uptake and transport;

As a new *in vitro* model of the respiratory mucosa, we developed a method to grow human alveolar epithelial type cells (HAEpC) on permeable filters in primary culture, forming a confluent monolayer with functional tight junctions (2). While at present no permanent cell line seems to be suitable to study drug transport at the *alveolar* epithelium, some cell lines derived from *airway* epithelial cells can be used for pharmaceutical investigations, depending on the culture conditions (3).
In order to improve the delivery of macromolecules, we are exploring the potential of specific bioadhesive ligands, in particular lectins and lectin-like molecules (4). Currently, we are using computer-aided modeling and docking, as well as atomic force microscopy to calculate and measure binding forces, respectively, relevant to the design of structurally optimized lectino-mimetics for controlled cellular targeting and delivery (5). Such ligands may be coupled to the surface of nanoparticles, prepared from lipids, polymers or inorganic materials, representing an alternative to viral gene vectors, and allowing targeted delivery of drugs to specific areas of mucosal epithelia.
Cationic silica nanospheres (SiNP) were obtained by covalent modification of commercial SiO_2 -colloids with aminoalkylsilanes1,2. The size could be varied between 10 and 100 nm and a positive zeta potential of more than +31 mV at pH 7.4 was be obtained (6, 7). Such cationically surface modified silica nanoparticles form stable complexes with DNA under physiological conditions and are able to transfect epithelial cells in vitro with marker genes. Observed Transfection rates were comparable to those observed with other non-viral gene transfer systems (e.g. pEI and other polymers.) Surprisingly, the cytotoxicity of the Silica-nanoparticles was much lower than for other systems, which underscores the favorable safety profile of this new technology platform for gene delivery.
Finally, the anti-inflammatory drug rolipram was nano-encapsulated in poly(lactic-co-glycolic acid) (PLGA) by an optimized oil-in-water emulsion technique (8) in order to demonstrate the potential of such nanoparticles for the targeted delivery to ulcerated areas of the colonic mucosa in a relevant animal model *in vivo*. After demonstrating size dependent bioadhesion of nanoparticles to the inflamed colonic mucosa (9), biodegradable PLGA-nanoparticles containing the anti-inflammatory drug rolipram were administered to TNBS-induced colitic rats. Compared to the same dose of the free drug (administered as solution), nanoparticle formulations lead to a prolonged amelioration of clinical effect scores and a reduction of adverse neurotropic effects (10).

References

1. Lehr, C. M. Cell Culture Models of Biological Barriers: In-vitro Test Systems for Drug Absorption and Delivery, Taylor and Francis, London, **2002.**
2. Elbert, K. J., Schäfer, U., Schäfers, H. J., Kim, K. J., Lee, V. H. L., Lehr, C.-M. Monolayers of human alveolar epithelial cells in primary culture for pulmonary absorption and transport studies. *Pharm. Res.* **1999**, *16*(5), 601-608.
3. Ehrhardt, C., Kneuer, C., Fiegel, J., Hanes, J., Schaefer, U.F., Kim, K.-J., et al. Influence of apical fluid volume on the development of functional cellular junctions in the human epithelial cell line 16HBE14o-: implications for its use as an in vitro model for bronchial drug absorption studies. *Cell and Tissue Research* **2002**, in press.

4. Haas, J., Lehr, C.-M. Developments in the area of bioadhesive drug delivery systems. *Exp. Op. Biol. Therapy* **2002**, *2*(3), 287-298.
5. Neumann, D., Kohlbacher, O., Lenhof, H. P., Lehr, C.-M. Lectin-sugar interaction: Calculated versus experimental binding energies. *Eur.J.Biochem*. **2002**, *269*(5):1518-24.
6. Kneuer, C., Sameti, M., Haltner, E. G., Schiestel, T., Schirra, H., Schmidt, H., et al. Silica nanoparticles modified with aminosilanes as carriers for plasmid DNA. *Int.J. Pharm*. **2000**, *196*, 257-261.
7. Kneuer, C., Sameti, M., Bakowsky, U., Schiestel, T., Schirra, H., Schmidt, H., et al. Surface modified silica-nanoparticles can enhance transfection in vitro: A novel class of non-viral DNA vectors. *Bioconj. Chem*. **2000**, *11*(6), 926-932.
8. Lamprecht, A., Ubrich, N., Yamamoto, H., Schäfer, U., Takeuchi, H., Lehr, C.-M., et al. Design of rolipram loaded nanoparticles: comparison of two preparation methods. *J.Control.Rel*. **2001**, *71*, 297-306.
9. Lamprecht, A., Schäfer, U., Lehr, C.-M. Size dependent bioadhesion of micro- and nanoparticulate carriers to the inflamed colonic mucosa. *Pharm. Res*. **2001**, *18*, 788-793.
10. Lamprecht, A., Ubrich, N., Yamamoto, H., Schäfer, U., Takeuchi, H., Maincent, P., et al. Biodegradable nanoparticles for the targeted drug delivery in treatment of inflammatory bowel disease. *J.Pharmacol. Exp. Therap*. **2001**, *299*(2), 775-781.

The Impact of Modern Biotechnology on the Pharmaceutical, Medical and Environmental Development

B. Štrukelj
Faculty of Pharmacy, University of Ljubljana, Slovenia
Jožef Stefan Institute, Ljubljana, Slovenia

Modern biotechnology or biological engineering has been defined in many different ways. By the expanded understanding and the combining of multiple disciplines such as molecular biology, genetics, immunology and genetic engineering, today's biotechnology has opened new perspectives for the future. With "classical" biotechnology, the selection of interest was based on visible characters of an organism. In modern biotechnology, new techniques allow us to detect and select genes of choice, even if their expression is not visible, and transfer them to the desired cell line in a very efficient and faster way. One of the most expanding fields in modern biotechnology is the development of biotech pharmaceuticals and biotherapy. On the other hand, through gene therapy, the potential of biopharmaceuticals for curing many diseases and improving the human condition is limitless. Since 1982, when the first recombinant drug-human insulin was manufactured, many new recombinant biopharmaceuticals, such us growth hormones, interferons, interleukins, erhytropoietin, glucagon, recombinant vaccines, streptokinase, filgrastim, sargramostim, alpha dornase, etc., have been approved and are currently on the market.
One of very interesting field in the creation of new biotech products are recombinant antibiotics. In order to find novel potent antibacterial agents, we construct a cDNA library from human and porcine bone marrow and isolate several clones, coding for antibacterial peptides. They possess a very potent antibacterial activity and thus might represent a new group of recombinant antibiotics.
At the Faculty of Pharmacy, two research groups are working in the area of osteoporosis. One of the medicines used for regulation of the bone remodeling cycle is hormone calcitonin. A synthetic gene, coding for human calcitonin, was synthesized and cloned. Some future aspects of the development of calcitonin expression and delivery systems will be discussed.
In the field of environmental and food biotechnology, a molecular biology group at Jozef Stefan Institute in collaboration with Krka Pharmaceutical Company cloned, elucidate the cDNA structure, patented and express a lamb chymosin, which is used as milk-clotting enzyme in cheese production. Later on, the pilot cheese making process by using expressed chymosin was successfully prepared. The same group, together with Dutch researchers, develops a transgenic insect-resistant potato plant. Beside environmental protection, the same potato plant system might serve as a bioreactor for producing pharmaceutically important recombinant proteins.

Helicobacter pylori Infection - Diagnosis and Treatment

K. G. Tchernev
Medical Faculty, Sofia, Bulgaria

Since its discovery in 1982, there is no doubt, that *Helicobacter pylori (H. pylori)* has emerged as the most important human gastroduodenal pathogen, being implicated in the pathogenesis of peptic ulcer disease (PUD), gastric cancer, gastric lymphoma and, possibly, other digestive conditions.
The initial diagnosis of *H. pylori* infection is made routinely by simultaneous agreement of histology and rapid urease test. The 13C-urea breath test (13C-UBT) as well as the fecal antigen determination by enzyme-linked immunosorbent assay (ELISA) (Hp-SA) is accepted as the gold-standard non-invasive tests in the initial diagnosis. After treatment, UBT should be the preferred test, since available data concerning the post-treatment accuracy of Hp-SA are fairly limited, so far.
For post-treatment evaluation, the simultaneous agreement of two of the following three tests, namely histology, rapid urease test and 13C-UBT are need. The “search and treat” strategy is recommended for PUD with long history, of the disease, in patients with gastro-esophageal reflux disease (GERD) requiring long-term acid suppression and in those taking long term non-steroidal anti-inflammatory drugs, as well as in areas with a high incidence of gastric cancer.
Treatment of the *H. pylori* infection with modern drug regimens is successful in over 80% of cases, with minor side effects, although bacterial resistance to antimicrobials is rapidly increasing.
H. pylori eradication has been strongly recommended by the Maastricht guidelines in all infected patients with present or past PUD documented at endoscopy, in patients with bleeding peptic ulcer, in patients with mucosa-associated tissue (MALT) lymphoma, severe gastritis, as well as cancer prevention after resection.
H. pylori eradication treatment should be no longer the domain solely of gastroenterologist.
Though the Maastricht indication, the first- line therapy should be a PPI-based triple therapy consisting of a PPI and two of the following antibiotics: clarithromycin 500 mg bid, amoxicillin 1 g bid or a nitroimidazole (metroimidazole or tinidazole 1 g/day) in various combination for seven days. Patients in whom this treatment fails may undergo eradication with second-line agents (choosing between antibiotics not previously used or quadruple therapy), or may undergo invasive testing and culturing for sensitivity analysis, in order to prescribe the appropriate antimicrobial therapy.

Metabolism of Drugs and Other Xenobiotics by Gut Microflora

M. Mikov
Medical Faculty, Novi Sad, FR Yugoslavia

Gut flora metabolic research within last decade has been expanded concerning different aspects of gut flora metabolic activity and proposed more fully determination of the diversity of the gut flora and the probiotic and prebiotic approach. The purpose of these studies has been to develop, refine, validate and apply molecular approaches for an improved qualitative and quantitative monitoring of the human intestinal microbiota providing scientific and industrial community with systematically evaluated methods for precise monitoring of the gut microflora.

It has been clearly proven for the last seven decades that xenobiotics could be inactivated, or activated to toxic or pharmacologically active products by the gut flora. In most cases it is not clear the benefit of the gut flora metabolic activity and secondary metabolite formation. Special attention has been paid to further xenobiotics glutathione conjugate metabolism as a toxic and carcinogenic pathway. The importance of gut flora metabolism for the bioavailability and drug effect has been shown. Gut flora metabolism studies should be the compulsory part of new and old drugs safety studies.

The complex gut microflora ecosystem is established at birth by colonization and nutrition. The differences between gut flora composition between breastfed and formula-fed full-term infants and premature neonates can be additional explanation for many differences in xenobiotic influence on infants and differences in comparison with adults.

Xenobiotics interfere in the gut contents ecology and it can be considered with the same methodological approach as in different ecosystem contamination investigations.

Nutrients and xenobiotics are interacting with these ecosystems and they are important for their alteration. Probiotics and prebiotics can modify composition and metabolic activity of these sophisticated ecosystems.

Variations within and between individuals in gut flora are so huge that no significant correlations were found between bacterial composition and factors like pH, moisture, enzyme activity and putrefactive products concentrations, but it is clear that multiple microorganism species may cooperate.

Nowadays the research is concentrated on gut flora modulation of gene expression importance for intestinal maturation and angiogenesis and intestinal functions like absorption, mucousal barrier establishment, immune system development and xenobiotics metabolism.

Metabolic transformations of different xenobiotics within colon contents by gut flora should be considered more important after findings that colon has important role in not only water and sodium absorption but also carbohydrates and medium chain fat absorption. Important fact is that colonic mucosa is unable to nourish itself from the blood and nutritive demand must be met from the lumen (short-chain fatty acids, amino acids, polyamines, growth factors, vitamins and antioxidants) produced by protective, “probiotic” flora from nutrients referred as prebiotics- fibers and proteins (colonic food) and from mucosal cells, mucus, secretions, bacteria and yeasts.

It is not only that xenobiotic metabolites produced by gut flora can influence the function of the liver and body but also bacterial constituents and products of bacteria metabolism (for example mitogens) can pass from the gut lumen to the portal system and influence the liver function.

One of methods to study gut flora role in metabolism and the possibility of its modification is by the use of human gut flora -associated rats, colonized with microflora originating from different human populations. The latest *in vitro* system for studying the metabolic interaction of xenobiotic metabolism of liver and faecal microflora developed by Laube et al. (*Arch. Toxicol.* **2000**, *74*, 379-387) could be a precious tool for the revealing answers about the role and importance of intestinal microflora and liver for xenobiotic metabolism.

In our studies on conventional, antibiotic pretreated, and germ free animals we clearly showed the importance of gut flora activity for paracetamol and verapamil metabolism through the *in vitro* and *in vivo* studies.

32.
Krkine
nagrade
32nd
Krka
Prizes

2 Krka Prize Winners´ Presentations

32

Aerobic and Anaerobic Life on Carbon Monoxide Oxidation

H. Dobbek
Max-Planck-Institut für Biochemie, Martinsried, Germany
Supervisor: R. Huber
Max-Planck-Institut für Biochemie, Martinsried, Germany

CO is a colorless, odorless gas, which is highly toxic to most forms of life. Despite its toxicity CO can be used by several bacteria and archaea as a chemolithoautotrophic growth substrate, providing these microbes with energy and a carbon source. CO dehydrogenases are the key enzymes in this process and catalyse the formal reaction: $CO + H_2O \rightarrow CO_2 + 2H^+ + 2e^-$. Two structurally unrelated principal types of CO dehydrogenases have been described.
The CO dehydrogenase from the aerobic CO-oxidizing eubacterium *Oligotropha carboxidovorans* is a 277-kDa Mo- and Cu-containing iron-sulfur flavoprotein. In my PhD work the enzyme´s active site in the oxidized or reduced state and after inactivation with potassium cyanide or n-butylisocyanide has been investigated by multiple wavelength anomalous dispersion measurements up to 1.09 angstrom resolution. We are able to present evidence for a binuclear heterometal [CuSMoOOH] cluster in the active site of the oxidized or reduced enzyme. The metals are bridged by a μ-sulfido ligand. The cluster is coordinated through interactions of the Mo with the dithiolate pyran ring of molybdopterin cytosine dinucleotide and of the Cu with the Sγ of cysteine388. The Cu ion as well as the μ-sulfido ligand react with cyanide yielding copper cyanide (CuCN) or thiocyanate (SCN^-), respectively. The structure of CO dehydrogenase with the inhibitor n-butylisocyanide bound has led to a model for the catalytic mechanism of CO oxidation which involves a thiocarbonate-like intermediate state.
The homodimeric nickel-containing CO dehydrogenase from the anaerobic bacterium *Carboxydothermus hydrogenoformans* catalyses the oxidation of CO to CO_2. A crystal structure of the reduced enzyme has been solved at 1.1 angstrom resolution. This structure represents the prototype for Ni-containing CO dehydrogenases from anaerobic bacteria and archaea. It contains five metal clusters of which clusters B, B´ and a subunit-bridging, surface-exposed cluster D are cubane-type [4Fe-4S] clusters. The active site clusters C and C´ are novel, asymmetric [Ni-4Fe-5S] clusters. Their integral Ni-ion, which is the likely site of CO oxidation, is coordinated by four sulfur ligands with square planar geometry.
The knowledge obtained from these two representative structures allows us now to gain further insight into the function and evolutionary relationships of CO dehydrogenases and related metalloproteins.

Nanoparticle Targeting as a Therapeutic Approach in Inflammatory Bowel Disease

A. Lamprecht
Saarland University, Saarbrücken, Germany
Supervisor: C.-M. Lehr
Saarland University, Saarbrücken, Germany

Introduction: In the case of inflammatory bowel disease, several symptoms are known to activate the cellular immune response in the surrounding tissue of ulcerations. This may used as a selective target for a particle uptake into ruptures or other strong damages of the mucosa by ulceration, which allows the accumulation of the particulate carrier system in this area. Such accumulation was observed in particular for sub-micron sized particles, which has been proven in an earlier study. This project elucidates the targeting potential of nanoparticles (NP) to the inflamed tissue *in vivo* in order to achieve local drug delivery. The therapeutic efficiency of this drug carrier system is evaluated in an experimental colitis rat model.
Methods: Rolipram has initially been developed as an antidepressant drug. The potential therapeutic use of this drug in tumor necrosis factor-α dependent diseases has been demonstrated in several animal models. Rolipram as an anti-inflammatory model drug was incorporated into poly[lactic-co-glycolic acid] (PLGA) NP by an oil-in-water emulsion technique. NP were separated from non-encapsulated drug, washed three times before lyophilization and finally redispersed in phosphate buffer at pH 6.8 prior to administration to rats.
Experimental colitis was induced with trinitrobenzenesulfonic acid in male Wistar rats (n=6 per group). The animals of each group received orally either rolipram solution or NP suspension, continuously for five days. Animals were sacrificed either 24 hours after the last drug/NP administration or after another five days without treatment. The severity of the inflammation was characterized by a clinical activity score and the myeloperoxidase activity (MPO). The neurotropic effect of rolipram was determined in order to quantify systemic absorption of the drug.
Results and discussion: After a lag time of 24 to 48 hours the clinical activity was lowered by drug solution and for the two NP groups during the treatment period. The five following days without drug treatment led to a strong relapse of the solution group, whereas for the NP groups a continuously reduced clinical activity score was observed. The neurotropic effect index was distinctly lowered when rolipram was incorporated into NP compared to free drug solution.
The clinical activity score and MPO activity decreased significantly after the oral administration of rolipram NP or drug solution. All NP formulations proved to be as efficient as the free drug in mitigating the experimental colitis. On the day of final drug administration, the differences between rolipram treated and control groups were dramatic, while those for the mitigating effect of all rolipram-receiving groups were not significant. 5 days after the last drug administration, the group initially treated with drug solution showed a distinct relapse, manifested by clinical activity score and MPO activity while the NP groups continuously maintained at a significantly reduced level. This suppressed relapse might be due to an accumulated deposition of NP in the inflamed tissue, which is in line with observations in an earlier study. On the other hand, the reduction of neurotropic effects after the administration of the rolipram NP might be due to the reduced availability of rolipram during the gastrointestinal passage. The remaining adverse effect, which was observed for both NP preparations, might be based on the initial burst effect as observed *in vitro*. This drug release in the stomach and small intestine is in favor to drug absorption from the gut and subsequent some remaining adverse effects of rolipram.
Conclusions: The targeted accumulation of nanoparticulate drug delivery systems in the inflamed colonic mucosal tissue represents a new and promising approach for the future development of modern drug carriers in the treatment of inflammatory bowel disease.

Gene Therapy of Coronary Artery Disease in Patients Undergoing Surgical Incomplete Myocardial Revascularization

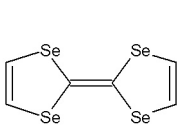
P. Kołsut
National Institute of Cardiology, Warsaw, Poland
Supervisor: Z. Religa
National Institute of Cardiology, Warsaw, Poland

Background: We initiated clinical study to determine safety and activity of gene encoding vascular endothelial growth factor (VEGF165) administered directly into the myocardium during coronary artery by pass grafting (CABG) operation.
Our trials with the plasmid encoding VEGF165 shows that intramyocardial application is safe and patients have benefited with the therapy.
Methods: VEGF gene transfer was performed in 15 patients (13 male, 2 female, ages from 50 to 73 years old). 200 μg of the plasmid encoding VEGF165 was injected into the ischaemic myocardium that could not be surgically revascularized. Patients with ejection fraction (%EF) < 30%, fertile females, acute myocardial infarction (MI), retinopathy and neoplasm were excluded from the study. Ten patients underwent CABG and gene therapy, in five cases there was not possibility of CABG because of surgical limitations and the application of genes encoding VEGF165 was the only solution.
%EF, perfusion of the myocardium, ECHO, angiogram, ventriculography, quality of life and nitroglycerin consumption were evaluated pre- and postoperatively.
Results: All patients tolerated surgery uneventfully and no fatal outcome was observed. There was no evident change in %EF or it improved at most 5 to 8 %. Nearly, all patients have been angina free for 6 months after surgery. Patients reported much better quality of life and reduction of nitroglycerin use. A reduction in ischaemic defects on single photon emission computerized tomography scans (SPECT) were observed. Significant improvement was seen particularly in rest SPECT scans. In some of patients angiographies revealed improved collateral filling.
Conclusions: Direct myocardial administration of genes encoding VEGF165 can be an effective method of treatment patients with chronic and advanced coronary artery disease either as a supplementary or as a sole therapy.

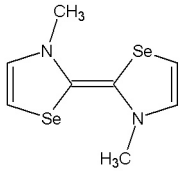
Diselenadiazafulvalenes: Novel Electron Rich Olefins Precursors of Organic Materials

Z. Časar
Université de Rennes 1, Rennes, France
Supervisor: D. Lorcy
Université de Rennes 1, Rennes, France
Co-supervisors: A Majcen Le Maréchal
Faculty of Mechanical Engineering, University of Maribor, Slovenia
M. Japelj
Krka, d. d., Novo mesto, Slovenia
A. Robert
Université de Rennes 1, Rennes, France

The main objective of this work is the synthesis of diselenadiazafulvalenes (DSeDAF), a novel family of π-donor molecules, and preparation of charge transfer salts based on these nitrogen analogues of tetraselenafulvalenes (TSF).

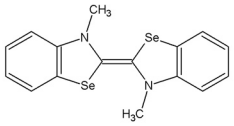


TSF

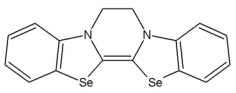


DSeDAF

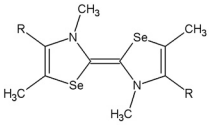
We present the first synthetic approach to the diselenadiazafulvalenes. Either N,N'-dimethyl dibenzoDSeDAF or N,N'-ethylene dibenzoDSeDAF are prepared via alkylation of 3H-benzoselenazole-2-thione core. Moreover, we show that diselenadiazafulvalenes are oxygen sensitive due to their excellent electron-donating ability. Introduction of an ethylene bridge between the two heterocyclic cores of the diselenadiazafulvalene framework significantly increases the potential domain of stability of the cation radical species. For the first time diselenadiazafulvalene is trapped in situ with TCNQ giving the DSeDAF•$(TCNQ)_3$ complex. Analysis of this complex shows that DSeDAF donor under the dicationic state is almost planar.
Furthermore, we describe the first approach to 2-alkylthio-1,3-selenazole core. These novel selenazoles afford 4,4',5,5'-tetrasubstituted-DSeDAF.



N,N'-dimethyl dibenzoDSeDAF

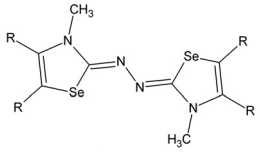


N,N'-ethylene dibenzoDSeDAF

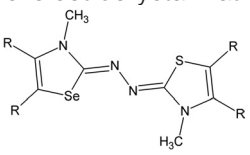


4,4',5,5'-tetrasubstituted-DSeDAF

Finally, we present the synthesis and of aza-vinylogous π-donors containing selenazole moieties: azino-diselenadiazafulvalenes (azino-DSeDAF) and azino-selenathiadiazafulvalenes (azino-SeTDAF). Azino spacer group induces an anodic shift of the oxidation potentials towards positive values as well as an increase of the potential window stability of the cation radical species. These donors afford neutral complexes with TCNQ and cation radical salts using the electrocrystallization technique.



azino-DSeDAF



azino-SeTDAF

Optotermične metode za raziskovanje vzorcev z visoko absorpcijo in v prisotnosti motečega sipanja

G. Močnik
Institut Jožef Stefan, Ljubljana
Mentor: M. Franko
Politehnika Nova Gorica, Nova Gorica, Slovenija
Somentor: D. D. Mihailović
Institut Jožef Stefan

Eksperimentalno sem preveril nov teoretični model, ki razlaga hkratni pojav termične leče in pojav odklona snopa. Raziskal sem vpliv odklona na meritve termične leče in vpliv termične leče na meritve odklona laserskega snopa. Eksperiment je potrdil veljavnost teoretičnih napovedi za meritve močno absorbirajočih vzorcev.
Preizkusil sem uspešnost dvožarkovnega spektrometra, delujočega na principu termične leče, za detekcijo absorpcije infrardeče svetlobe z valovno dolžino v območju delovanja CO laserja v tekočinskem kromatografskem sistemu. Preučil sem vpliv močne absorpcije ozadja in toka skozi kromatografsko celico na signal termične leče. Mono in večkrat nenasičene maščobne kisline v testni mešanici sem ločil brez derivatizacije. Podoben eksperiment sem naredil še z metil estri maščobnih kislin. Rezultate nove detekcijske sheme sem primerjal s tistimi, ki sem jih dobil z detektorjem lomnega količnika in detektorjem absorpcije ultravijolične svetlobe. Prikazal sem prednosti detekcije s termičnimi lečami, ko poskušamo zaznavati snovi, ki se v koloni ne ločijo, kot so maščobne kisline in alkoholi.
Določil sem globinske profile barvil v silikagelu na ploščah za tenkoplastno kromatografijo. Toplotno difuzivnost in efuzivnost silikagela sem dobil s sunkovno optotermično radiometrijo. Z novo inverzijsko metodo sem dobil globinske profile. Večina barvila leži v zgornji polovici silikagela.

Photothermal Methods for Investigation of Highly Absorbing and Scattering Samples

G. Močnik
Jožef Stefan Institute, Ljubljana, Slovenia
Supervisor: M. Franko
Nova Gorica Polytechnic, Nova Gorica, Slovenia
Co-supervisor: D. D. Mihailović
Jožef Stefan Institute, Ljubljana, Slovenia

We experimentally tested a newly developed theoretical model describing simultaneous thermal lens and beam deflection phenomena. The effect of both beam deflection on thermal lens measurements and that of the thermal lens on beam deflection measurements were investigated. The experiment confirmed the validity of theoretical predictions in studies of strongly absorbing samples.
The potential of a dual beam thermal lens spectrometry used as a detector of the infrared absorption behind reversed-phase HPLC column was investigated at CO laser wavelengths for the first time. Feasibility study of the thermal lens detection in a flowing medium with high background absorption was carried out. Mono- and polyunsaturated fatty acids in the model mixture were resolved without the need for their derivatization. We conducted a similar experiment for fatty acid methyl esters. The performance of the novel scheme was compared to that obtainable by the refractive index detector and the UV absorbance detector. The advantage of the thermal lens detection, when coeluting compounds such as fatty acids and alcohols are present, was demonstrated for the first time.
We determined the depth profiles of dyes in the silica-gel on thin-layer chromatography plates. We performed pulsed photothermal radiometric measurements to obtain the thermal diffusivity and effusivity of the silica gel. The newly proposed inversion of the data collected using photoacoustic measurements with modulated excitation yielded the depth profiles. Most of the dye was found within the upper half of the silica-gel layer.

Reaktivni intermediati pri ozonizaciji nasičenih ogljikovodikov

E. Eržen
Fakulteta za kemijo in kemijsko tehnologijo, Univerza v Ljubljani, Slovenija
Mentor: B. Plesničar
Fakulteta za kemijo in kemijsko tehnologijo, Univerza v Ljubljani, Slovenija

Pri nizkotemperaturni ozonizaciji serije nasičenih "metinskih" in "metilenskih" ogljikovodikov ter sekundarnih alkoholov v acetonu-*d6*, metil acetatu in *t*-butil metil etru nastajajo ustrezni alkil hidrotrioksidi oziroma hidroksihidrotrioksidi. Pri ozonizaciji ogljikovodikov, ki imajo na razpolago abstraktabilen β vodik, in pri ozonizaciji sekundarnih alkoholov nastaja tudi vodikov trioksid (H_2O_3). Le-tega pa ni mogoče najti pri ozonizaciji trifenilmetana (Ph_3CH). Navedene eksperimentalne ugotovitve se skladajo s predlaganim "radikalskim" mehanizmom prve stopnje ozonizacije C-H vezi, pri kateri nastane radikalski par (R••OOOH) v "kletki" topila. Le-ta lahko rekombinira v alkil hidrotrioksid (ROOOH), lahko pa pride do abstrakcije β vodika z alkilnega radikala kar vodi do nastanka alkena in H_2O_3. Pri sekundarnih alkoholih je na razpolago tudi alternativna pot: hidrotrioksilni radikal abstrahira vodik iz -OH skupine; nastaneta keton in H_2O_3.
"Metinski" alkil hidrotrioksidi in geminalni hidroksihidrotrioksidi razpadejo v "kletki" topila po radikalskem mehanizmu s cepitvijo RO-OOH vezi. Nasprotno pa "metilenski" hidrotrioksidi razpadajo po "pericikličnem" mehanizmu z abstrakcijo geminalnega vodika in nastankom ketona in vodikovega peroksida (H_2O_2).

The Reactive Intermediates in the Ozonation of Saturated Hydrocarbons

E. Eržen
Faculty of Chemistry and Chemical Technology, University of Ljubljana, Slovenia
Supervisor: B. Plesničar
Faculty of Chemistry and Chemical Technology, University of Ljubljana, Slovenia

Low-temperature ozonation of various saturated "methyne" and "methylene" hydrocarbons and secondary alcohols in acetone-*d6*, methyl acetate and *t*-butyl methyl ether produced the corresponding hydrotrioxide or hydroxyhydrotrioxide. In case of abstractable β hydrogen atom hydrogen trioxide (H2O3) is produced as well. H_2O_3 was also formed during low-temperature ozonation of secondary alcohols. Ozonation of triphenylmethane (Ph_3CH) produced only triphenylmethyl hydrotrioxide (Ph_3COOOH) but no H_2O_3. These observations are in agreement with previously suggested "radical" mechanism for the first step of the ozonation of the C-H bonds, i.e. the formation of the caged radical pair (R••OOOH). This radical pair is capable of recombination to alkyl hydrotrioxide (ROOOH) or the abstraction of β hydrogen atom to form the corresponding alkene and H_2O_3. Another reaction pathway is also available for secondary alcohols, i.e. hydrotrioxyl radical can abstract hydrogen from hydroxy group to produce the corresponding ketone and H_2O_3.
The decomposition of "methyne" alkyl hydrotrioxides and geminal hydroxyhydrotrioxides involves a homolytic cleavage of the RO-OOH bond inside the solvent "cage". The decomposition of "methylene" alkyl hydrotrioxides on the other hand is most likely a "perycyclic" process involving the abstraction of the geminal hydrogen with generation of ketone and hydrogen peroxide (H_2O_2).

Sinteze in pretvorbe stabilnih heteroarildiazonijevih soli

S. Rečnik
Fakulteta za kemijo in kemijsko tehnologijo, Univerza v Ljubljani, Slovenija
Mentor: J. Svete
Somentor: B. Stanovnik
Fakulteta za kemijo in kemijsko tehnologijo, Univerza v Ljubljani, Slovenija

Pri preučevanju sintez in pretvorb skupine stabilnih heteroarildiazonijevih soli, npr. 4-oksokinolizin-3-diazonijevega tetrafluoroborata, njegovih aza analogov ter 3-azido derivatov so se le-ti izkazali kot učinkoviti in raznovrstni prekurzorji v sintezi nekaterih heterocikličnih sistemov, po drugi strani pa gre pri reakcijah teh sistemov mnogokrat za mehanistično zanimive in nenavadne pretvorbe. Tako smo pri reakcijah 1-substituiranih 4-okso-*4H*-kinolizin-3-diazonijevih tetrafluoroboratov v alkoholnih medijih opazili aza Wolffovo premestitev v indolizin-3-karboksilate (kar je prva opisana Wolffova premestitev kondenzirnih α-diazoamidov), medtem ko so pri reakcijah 4-okso-*4H*-piridino[1,2-*a*]pirimidin-3-diazonijevih tetrafluoroboratov prav tako v alkoholnih medijih nastali alkil 1-(piridin-2-il)-*1H*-1,2,3-triazol-4-karboksilati. Pri reakcijah s spojinami z aktivnimi metilenskimi skupinami smo izolirali hidrazone, nekatere med njimi pa ciklizirali v 1-heteroaril-*1H*-pirazol-3-karboksilate. S sekundarnimi amini so nastali 1-heteroaril-3,3-dialkil triazeni, s primarnimi amini pa je pri ostrejših pogojih prišlo do premestitve v piridinkarboksamide. Pri termičnih reakcijah 3-azido derivatov so potekale skrčitve obročev in nastali so substituirani 3-amino indolizini, imidazo[1,2-*a*]piridini in imidazo[1,2-*b*]piridazini. Pri reakcijah kondenziranih 3-diazopiran-2-onov pa smo podobno kot v kinolizinonski seriji opazili hetero-Wolffove premestitve in nastanek furo[2,3-*d*]pirimidinov.

Syntheses and Transformations of Stable Heteroaryl-diazonium Salts

S. Rečnik
Faculty of Chemistry and Chemical Technology, University of Ljubljana, Slovenia
Superisor: J. Svete
Co-supervisor: B. Stanovnik
Faculty of Chemistry and Chemical Technology, University of Ljubljana, Slovenia

A series of stable heteroaryldiazonium salts (derived in high yields from dimethylaminopropenoates), e.g. 4-oxoquinolizine-3-diazonium tetrafluoroborates, its aza analogues, and 3-azido derivatives were developed as highly versatile and efficient precursors in the synthesis of several heterocyclic systems, while on the other hand, these compounds underwent some mechanistically interesting and unusual transformations. Alkyl 1-heteroaryl-*1H*-1,2,3-triazole-4-carboxylates were obtained in a "ring-switching" rearrangement, while 1-substituted indolizine-3-carboxylates were formed in a novel aza Wolff rearrangement. The latter reaction represents the first documented Wolff rearrangement of fused cyclic α-diazoamides. Additionally, 1-heteroaryl-*1H*-pyrazole-3-carboxylates were obtained from the corresponding diazonium salts via heterocyclic hydrazones, regioselectively. In reactions of 4-oxoquinolizine-3-diazonium salt with aliphatic amines, 1-heteroaryl-3,3-dialkyl triazenes were formed in excellent yields upon treatment with secondary amines. On the other hand, in reactions with primary amines at reflux temperature, transformation into unexpected products - pyridinecarboxamides took place. Furthermore, upon reactions of 3-azido derivatives in acetic anhydride and in acetic acid, ring contraction products - substituted 3-aminoindolizines, imidazo[1,2-*a*]pyridines, and imidazo[1,2-*b*]-pyridazines were formed in high yields. Last but not least, in reactions of fused 3-diazopyran-2-ones, once again hetero-Wolff rearrangement was observed, leading to formation of ring contraction products, i.e. furo[2,3-*d*]pyrimidines.

Energetsko - snovne rekonstrukcije kompleksnih kontinuirnih procesov

A. Kovač Kralj
Fakulteta za kemijo in kemijsko tehnologijo, Univerza v Mariboru, Slovenija
Mentor: P. Glavič
Somentor: Z. Kravanja
Fakulteta za kemijo in kemijsko tehnologijo, Univerza v Mariboru, Slovenija

Razvili smo teoretično metodo za optimiranje zahtevnih procesov - večstopenjsko simultano metodo rekonstruiranja. Metoda omogoča zmanjšanje porabe energije in ekonomičnejše delovanje procesa. Sestavljajo jo tri stopnje: razvijanje superstrukture z analizo uščipa, oblikovanje modela z mešanim celoštevilskim nelinearnim programiranjem (MINLP), ki ga poenostavimo v model nelinearnega programiranja (NLP) in simultano optimiranje s programom ASPEN PLUS in z modelom NLP.
Razvijanje superstrukture je osnovano na termodinamskih načelih analize uščipa, s katero dobimo veliko možnih struktur za racionalno porabo energije.
NLP strukturo izbira s cepilniki tokovnic in s tem poenostavi problem do te mere, da postane rešljiv pri kompleksnih procesih. Prva in druga stopnja predstavljata poenostavljeno superstrukturo, ki omogoča reševanje kompleksnih procesov. Simultano parametrično in strukturno optimiranje s programom NLP daje najboljše rešitve.
Metodo smo preizkusili na nizkotlačni Lurgijevi shemi (slovenskega proizvajalca) proizvodnje metanola z velikim dodatnim letnim dobičkom. Ugotovljena je možnost povečanja stopnje presnove metanola, večji proizvodnji visoko-tlačne pare in nizko-tlačne pare, zmanjšanje porabe vode.
Razvili smo tudi teoretično metodo integriranja odpadne toplote med procesi. Z integracijo med procesi lahko zmanjšamo porabo energije in procesi obratujejo z večjim prihrankom. Uporabnost metode je ilustrirana na štirih zahtevnih slovenskih obratih z zadovoljivim dodatnim letnim dobičkom.

Energy - Mass Retrofit of Complex Continuous Processes

A. Kovač Kralj
Faculty of Chemistry and Chemical Technology, University of Maribor, Slovenia
Supervisor: P. Glavič
Co-supervisor: Z. Kravanja
Faculty of Chemistry and Chemical Technology, University of Maribor, Slovenia

We have developed the theoretical method for the optimization of complex processes - stepwise simultaneous structural and parameter optimization of retrofits. Method can reduce energy and give more economical operation. It is composed of three steps: generation of a process superstructure by pinch analysis, formulation of the mixed integer nonlinear programming (MINLP) model and its relaxation into the corresponding nonlinear programming (NLP) model, and simultaneous optimization using process simulator optimization and the NLP model.
The alternatives of energy structure are obtained by pinch analysis.
The NLP model of the superstructure uses splitters and mixers to simplify the problem solution. Both steps synthesize the superstructural approach for nontrivial processes. Structure and parameters are optimized using the NLP model and it can give good solutions.
The approach was tested on theoretical, complex, low-pressure Lurgi methanol plant (operated in Slovenia), which has the substantial additional annual profit. Additional quantities of methanol, high and low-pressure steam are supposed to be produced, while the quantities of cooling water should be reduced.
We had developed the theoretical method for waste heat integration of several processes. The integration between processes can reduce energy use and it can operate more economically.
The approach has been illustrated on four existing complex Slovenian processes with large additional annual profit.

Karakterizacija polimorfnih oblik doksazosinijevega mesilata in njegove amorfne faze

M. Grčman
Fakulteta za kemijo in kemijsko tehnologijo, Univerza v Ljubljani, Slovenija
Mentor: A. Meden
Fakulteta za kemijo in kemijsko tehnologijo, Univerza v Ljubljani, Slovenija
Somentor: F. Vrečer
Krka, d. d., Novo mesto, Slovenija

V okviru dela smo identificirali posamezne polimorfne oblike doksazosinijevega mesilata (DM) in njegovo amorfno obliko ter določili temperaturne intervale stabilnosti za posamezno obliko.
Pri eksperimentalnem delu smo uporabili termično analizo (TGA in DSC), rentgensko praškovno difrakcijo, FT-IR spektroskopijo, termomikroskopijo in SEM analizo ter analizo raztapljanja vzorcev. Posameznim vzorcem smo določili tudi velikost in specifično površino delcev ter močljivost na osnovi merjenja stičnih kotov vode.
Polimorfni obliki A in F sta stabilni v temperaturnem območju od sobne temperature do njunih tališč. Oblika D izkazuje ireverzibilen polimorfen prehod trdno-tekoče-trdno v obliko I. Polimorfna oblika H s segrevanjem nad temperaturo okoli 240^{O}C ireverzibilno prehaja v polimorfno obliko F. Amorfna oblika, ki je stabilna pri sobnih pogojih, nad temperaturo steklastega prehoda kristalizira v zmes najmanj dveh polimorfnih oblik. V nastali zmesi smo identificirali novo polimorfno obliko E, ki pa pri nadaljnjem segrevanju prehaja v obliko F. Psevdopolimorfna oblika G in amorfna oblika sta higroskopni. SEM mikrofotografije kažejo na različno morfologijo delcev analiziranih vzorcev. Polimorfna oblika A, oblika z najvišjim talíščem, ima najvišjo relativno hitrostno konstanto raztapljanja (k), amorfna oblika pa najnižjo. Navedene rezultate smo pojasnili na osnovi rezultatov merjenja stičnega kota vode oz. močljivosti vzorcev.

Characterization of Polymorphic Forms of Doxazosin Mesylate and Its Amorphous Phase

M. Grčman
Faculty of Chemistry and Chemical Technology, University of Ljubljana, Slovenia
Supervisor: A. Meden
Faculty of Chemistry and Chemical Technology, University of Ljubljana, Slovenia
Co-supervisor: F. Vrečer
Krka, d. d., Novo mesto, Slovenia

In the present study, the polymorphism of doksazosin mesylate (DM), together with the temperature ranges of stability of the relevant crystalline forms and of the amorphous form has been examined.
The DM polymorphs were studied by thermal analysis (TGA and DSC), X-ray powder diffractometry, FT-IR spectroscopy, hot stage and scanning electron microscopy and by dissolution measurements. Some of the samples were also studied by particle size measurements, specific surface determinations and by determining the wettability by contact angles measurements.
Polymorphs A and F were found to be stable from room temperature to their melting points. Form D undergoes irreversible solid-liquid-solid phase transition to polymorph I. Form H is irreversibly transformed to polymorph F during heating above the temperature of about 240^{O}C. The amorphous form was found to be stable at room temperature, but when heating above the glass transition, it crystallizes, what leads into a mixture of at least two polymorphic forms. The new polymorphic form designed as E was identified in the mixture. The polymorphic form E is converted by heating to the more stable form F. The pseudo-polymorphic form G and the amorphous form are hygroscopic. SEM micrographs showed different morphologies between analysed samples. The highest melting polymorph A shows the highest dissolution rate constant (k) and the amorphous form has the lowest one.

Procesna separacija in določevanje metabolitov bioprocesa na monolitnih nosilcih

M. Vodopivec
Kemijski inštitut, Ljubljana, Slovenija
Mentor: M. Berovič
Fakulteta za kemijo in kemijsko tehnologijo, Univerza v Ljubljani, Slovenija
Somentor: A. Podgornik
BIA Separations, d.o.o., Ljubljana, Slovenija

CIM nosilci so predstavniki novih kromatografskih materialov, ki omogočajo hitrejši proces ločitve v primerjavi s klasičnimi kromatografskimi kolonami. Uporabna vrednost CIM kromatografskih enot se je potrdila v primeru separacij velikih biomolekul, rezultati raziskav v pričujočem delu pa potrjujejo, da je na CIM nosilcih dejansko izvedljiva izokratska separacija tudi tako majhnih molekul kot so organske kislin ter, da tudi v tem primeru ne pride do transportnih omejitev kromatografskega procesa. Enako se izkaže v primeru uporabe CIM nosilcev kot encimskih reaktorjev, saj dejstvo, da so kinetični parametri imobiliziranega encima od pretoka neodvisni, potrjuje, da je v CIM encimskih sistemih difuzijski upor majhen in ne vpliva na celokupno hitrost encimske reakcije. Kot taki so CIM nosilci primerno orodje za izvedbo natančnih študij encimske kinetike imobiliziranih encimov.
Z uporabo CIM nosilcev sem razvila HPLC metodo za separacijo piruvata, malata, fumarata, α-ketoglutarata, izocitrata in citrata na CIM QA koloni ter v nadaljevanju, kot alternativne metode za določanje kislin, še FIA encimske metode za določanje citrata, izocitrata in piruvata, z uporabo CIM nosilcev kot encimskih reaktorjev. Postavljeni HPLC in FIA analitski tehniki sem opredelila s stališča primerjave rezultatov analize realnih vzorcev bioprocesa kvasovke *Yarrowia lipolytica*, kot tudi s stališča primerjave učinkovitosti metod ter ekonomske plati njune uporabe.

Process Separation and Determination of Bioprocess Metabolites on Monolithic Support

M. Vodopivec
National Institute of Chemistry, Ljubljana, Slovenia
Supervisor: M. Berovič
Faculty of Chemistry and Chemical Technology, University of Ljubljana, Slovenia
Co-supervisor: A. Podgornik
BIA Separations, d.o.o., Ljubljana, Slovenia

CIM support is a representative of new chromatographic materials in which the transport of molecules to the active groups is governed by convective flow. Consequently, much faster separation process can be achieved in comparison to classical chromatographic columns. The applicability of the CIM support for separations of biomolecules has already been described extensively, however, the results of the present research reveal that isocratic separation of small molecules such as organic acids can also be accomplished and that the separation process is not limited by mass transport. Just the same was observed when the CIM support was used as enzyme reactor. Namely, flow unaffected kinetic parameters of immobilised enzyme confirm that the overall rate of enzyme reaction in the CIM enzyme systems is not limited by diffusion. Due to this characteristic, the CIM support can represent a usefull toll for kinetic studies of the immobilised enzymes.
Using the CIM support, HPLC method for separation of pyruvate, malate, fumarate, α-ketoglutarate, isocitrate and citrate on CIM QA column was developed. On the other hand, FIA technique as alternative technique for organic acids determination was applied and FIA enzymatic methods for detection of pyruvate, citrate and isocitrate were developed, using the CIM support as the enzyme reactor. A detailed comparison of the developed HPLC and FIA analitical techniques is described with regard to analysis results of the real samples from bioprocess broth of yeast *Yarrowia lipolytica*, as well as in view of methods effectivness and economical side of their application.

Miš kot modelni organizem za študij vloge gena *Cyp51* v spermatogenezi

N. Debeljak
Medicinska fakulteta, Univerza v Ljubljani, Slovenija
Mentor: M. Rassoulzadegan
INSERM 470, Univerza v Nici-Sophia Antipolis, Francija
Somentorica: D. Rozman
Medicinska fakulteta, Univerza v Ljubljani, Slovenija

Okarakterizirali smo novega člana družine CYP51, mišjo lanosterolno 14α-demetilazo (*Cyp51*). Mišji gen *Cyp51* obsega ~23 kb in je sestavljen iz desetih eksonov, ki kodirajo 503 aminokislin velik protein. Sesalski geni CYP51 so visoko ohranjeni s podobno organizacijo gena, ohranjeno promotorsko regijo ter nad 91% aminokislinsko identičnostjo. Primerjava vseh znanih CYP51 proteinov nakazuje na skupen izvor glivnih in živalskih CYP51 ter rastlinskih in bakterijskih CYP51. Mišji genom vsebuje eno kopijo gena *Cyp51*, ki leži na področju A2 kromosoma 5, v bližini centromere ter ne vsebuje *Cyp51* procesiranih psevdogenov.
Za osvetlitev *in vivo* vloge gena *Cyp51* v reprodukciji sesalcev smo z uporabo tehnologije Cre-loxP začeli s pripravo pogojno ničelne miši, ki bo imela gen *Cyp51* izničen le v moških spolnih celicah. Popolno izničenje gena *Cyp51* bi najverjetneje bilo za zarodek smrtno, ker je gen vključen v biosintezo holesterola. Pripravili smo tri konstrukte $Cyp51^{loxP}$, ki vsebujejo loxP- ali Frt- omejeno *neo* selekcijsko kaseto ter dve mesti loxP, vključeni v introna 2 in 4 gena *Cyp51*. LoxP omejeni fragment *Cyp51* (vsebujoč introne 2-4) bo ob prisotnosti encima Cre izrezan, kar bo vodilo v zamik bralnega okvira in nastanek nefunkcionalnega proteina *Cyp51*.
LoxP-neo $Cyp51^{loxP}$ konstrukt smo elektroporirali v embrionalne klične (ES) celice WW6, kjer se med procesom homologne rekombinacije mesta loxP in loxP-omejena *neo* kaseta prenesejo v *Cyp51* ES celic. Med več kot 180 pregledanimi kolonijami celic ES nobena ni bila pozitivna za homologno rekombinacijo. V delu gena *Cyp51*, ki je bil vključen v konstrukt, leži 1-kb velika repetitivna regija LINE-1 (L1). Predvidevamo, da prisotnost L1 zmanjšuje uspešnost homologne rekombinacije v predelu *Cyp51*.

Mouse as a Model Organism to Study the Role of the *Cyp51* Gene in Spermatogenesis

N. Debeljak
Faculty of Medicine, University of Ljubljana, Slovenia
Supervisor: M. Rassoulzadegan
INSERM U470, University of Nice-Sophia Antipolis, France
Co-supervisor: D. Rozman
Faculty of Medicine, University of Ljubljana, Slovenia

A new member of the CYP51 family, murine lanosterol 14α-demethylase (*Cyp51*), has been characterised. The mouse *Cyp51* gene is ~23-kb long with 10 exons encoding a 503 amino acids long protein. Mammalian CYP51 genes are highly conserved with similar gene organisation, conserved promoter region and over 91% amino acid identity. Comparison of all known CYP51 proteins suggests that fungal and animal CYP51 genes and plant and bacterial CYP51 genes share a common progenitor. Murine genome contains a single copy of the mouse *Cyp51* gene residing on chromosome 5, region A2, close to the centromere and does not contains *Cyp51* processed pseudogenes.
To address the *in vivo* role of the *Cyp51* gene in mammalian reproduction we used the Cre-loxP strategy to develop a *Cyp51* male germ-cell specific knockout mouse. A total knockout would most probably be lethal as *Cyp51* is involved in cholesterol biosynthesis. Three $Cyp51^{loxP}$ recombinant targeting vectors containing loxP- or Frt-flanked *neo* selection cassette and two isolated loxP sites in introns 2 and 4 were prepared. The loxP-flanked *Cyp51* fragment (containing introns 2-4) will be deleted after expressing Cre enzyme, which should lead to a shift of the reading frame and production of nonfunctional *Cyp51*.

The *loxP-neo Cyp51*^{loxP} construct was electroporated in the WW6 embryonic stem (ES) cell line where loxP sites and loxP-flanked *neo* cassette are introduced in the ES *Cyp51* by the process of homologous recombination. Screening of more than 180 ES cells for homologous recombination did not give any positive colony. An 1-kb long repetitive element LINE-1 (L1) was found to reside in *Cyp51* region that was included into the targeting construct. We propose that presence of L1 reduced the efficiency of homologous recombination at the *Cyp51* site.

Mehanizem kooperativnih pojavov pri holinesterazah

M. Goličnik
Medicinska fakulteta, Univerza v Ljubljani, Slovenija
Mentor: J. Stojan
Medicinska fakulteta, Univerza v Ljubljani, Slovenija

Holinesteraze (HE) so hidrolitični encimi, odgovorni za razgradnjo acetilholina in drugih holinskih estrov v centralnem živčnem sistemu. Kinetične študije pokažejo zelo nenavadno delovanje. Pri različnih HE sta to bolj ali manj izrazita homotropična aktivacija in inhibicija.
S pričujočim delom smo želeli podrobneje proučiti naravo omenjene kooperativnosti pri različnih HE ter izdelati ustrezne metode za pridobivanje, obdelavo in razlago kinetičnih detajlov posameznega encima. Izvedli smo vrsto kinetičnih poskusov na točkovno mutiranih mutantah acetilholinesteraz (AHE) iz vinske mušice in človeških butirilholinesterazah (BHE). Proučevali smo tudi nemutirano naravno BHE iz konjskega seruma. Kot sonde za raziskovanje žepa v aktivno mesto encimov smo uporabili različne reverzibilne inhibitorje. V poskusih, kjer smo proučevali acilacijo, pa smo uporabili tudi ireverzibilni inhibitor. Pri razvoju novih eksperimentalnih in analiznih pristopov smo uporabili AHE iz električne jegulje zaradi relativno preproste kinetike tega encima v primerjavi z ostalimi.
V delu smo ugotovili: (i) da je pojav substratne aktivacije mogoče razložiti tudi le s substratno inhibicijo; (ii) da na kooperativne pojave pri HE lahko vplivajo tudi sterični efekti, a jih samo z njimi in brez alosterije ni mogoče razložiti; (iii) da se substrat najprej veže na periferno mesto, vendar je to mesto prvega zaznavnega kontakta pri različnih HE na različnih delih molekule; (iv) da zaradi perifernega mesta HE kažejo kooperativne pojave; (v) da se na periferno mesto naravne in mutiranih AHE iz vinske mušice veže substrat na prosti encim z visoko afiniteto tudi v primeru, ko substratna aktivacija ni dobro vidna; (vi) da lahko periferni reverzibilni inhibitor pospeši nastanek kovalentnega kompleksa encim-ireverzibilni inhibitor na račun izboljšane afinitete inhibitorja do encima, ne pa zaradi povečanja hitrosti nastanka kovalentne vezi.
Uporabljene analizne metode so v primerjavi s klasičnimi metodami hitrejše in natančnejše pri razločevanju posameznih reakcijskih mehanizmov, vrednosti kinetičnih parametrov pa so bolj verodostojne.

Mechanism of Cooperative Phenomena by Cholinesterases

M. Goličnik
Faculty of Medicine, University of Ljubljana, Slovenia
Supervisor: J. Stojan
Faculty of Medicine, University of Ljubljana, Slovenia

Cholinesterases (ChE) are hydrolytic enzymes responsible for metabolism of acetylcholine and other choline esters in central nervous system and in case of vertebrates also in other extracellular fluids.
The cooperative phenomena by various ChE have been studied in this work. Our aim was also to develop specific experimental and analytical methods for collecting kinetic data of each individual studied enzyme.
Experiments were carried out with various Drosophila melanogaster AChE and human BChE mutants obtained by site directed mutagenesis. Besides the wild type horse serum BChE has been also studied. Various reversible inhibitors have been applied as probes for kinetic explorations of enzyme active sites. The first step of catalytic process known as acylation of active serine has been investigated by using an ireversible inhibitor. Electric eel AChE with relatively simple kinetic behaviour has been used for the development of new experimental and analysis methods.

We found out that: (i) the phenomena of substrate activation could be explained also with substrate inhibition, (ii) the cooperative phenomena could be affected by steric hindrance but could not be explained without allostery, (iii) the first contact between the substrate and ChE occurs at the peripheral site but not at the same place in all enzymes, (iv) the peripheral site is responsible for the cooperative phenomena, (v) the wild type and various Drosophila AChE mutants bind the substrate to the peripheral site of free enzyme with high affinity even if substrate activation is not obvious; (vi) the peripheral reversible inhibitor accelerate the formation of covalent enzyme-ireversible inhibitor complex as a consequence of the reversible inhibitor affinity enhancement for small ireversible inhibitor rather to speeding up the formation of a stable covalent enzyme-inhibitor complex.
The applied analytical methods has been demonstrated as faster and more appropriate for the discrimination of individual possible reaction mechanism in comparison with common classical kinetic methods. The values of individual kinetic parameters evaluated in this manner are also more reliable.

32.
Krkine
nagrade
32nd
Krka
Prizes

3 Krka Prize Winners´ Poster Session

Določanje površinskih lastnosti laktoze z metodo inverzne plinske kromatografije

M. Barič, J. Jerman
Fakulteta za farmacijo, Univerza v Ljubljani, Slovenija
Mentor: O. Planinšek
Fakulteta za farmacijo, Univerza v Ljubljani, Slovenija

V farmacevtski tehnologiji želimo poznati površinsko energijo snovi, ki jih uporabljamo. V preteklosti smo v ta namen najpogosteje uporabljali metodo močenja, pri kateri merimo stični kot različnih tekočin z znano površinsko napetostjo in polarnostjo. Alternativo predstavlja inverzna plinska kromatografija (IGC-Inverse Gas Chromatography). Površinska energija kateregakoli praška, določena z inverzno plinsko kromatografijo, naj bi bila neodvisna od pogojev merjenja in uporabljenega tipa kolone. V nalogi smo z laktoznim praškom napolnili steklene kolone in kolone iz nerjavečega jekla. Določali smo nepolarno in polarno komponento površinske energije pri različnih pretokih nosilnega plina in pri uporabi laktoze kot vzorca v nerazredčeni obliki in razredčeno s Chromosorb W. Ugotovili smo, da pogoji merjenja ne vplivajo na nepolarni parameter površinske energije. Nasprotno pa sta vrednosti Ka in Kb, ki predstavljata kislo-bazične lastnosti površine trdne snovi, odvisna od redčenja s Chromosorb W. Vpliv ostalih spremenljivk na rezultat se je izkazal za manjšega. Z IGC smo ugotovili, da se laktoza monohidrat v interakcijah z ostalimi učinkovinami in pomožnimi snovmi obnaša kot elektron akceptor oz. Lewisova kislina.

Determination of Surface Properties of Lactose with Inverse Gas Chromatography

M. Barič, J. Jerman
Faculty of Pharmacy, University of Ljubljana, Slovenia
Supervisor: O. Planinšek
Faculty of Pharmacy, University of Ljubljana, Slovenia

In pharmaceutical technology we want to determine the surface energy of solid substances used in formulation production. In the past, Wilhelmy plate method was most often used method, measuring contact angle of different fluids with known surface tension and polarity. The inverse gas chromatography represents an alternative to Wilhelmy plate method. Surface energy of any powder determined by IGC should be independent on the measurement conditions or type of column used. In this study stainless steel and glass columns were filled with lactose powder. Dispersive and polar components of surface free energy were determined at different flow rates of carrier gas and by use of lactose in undiluted and diluted form with Chromosorb W. It was found that measurement conditions do not influence dispersive parameter of surface energy. On the contrary Ka and Kb values, representing acid-basic properties of solid surfaces, are dependent on dilution of lactose with Chromosorb W. Influence of all other parameters on the results were found to be much smaller. It was found that lactose monohydrate behaves in the interactions with other inactive and active ingredients as electron acceptor and Lewis acid respectively.

Optimizacija bioreaktorske proizvodnje ursolne kisline v žajblju (*Salvia officinalis* L.)

Ž. Bolta
Biotehniška fakulteta, Univerza v Ljubljani, Slovenija
Mentorica: D. Baričevič
Biotehniška fakulteta, Univerza v Ljubljani, Slovenija

Da bi lahko ocenili potencial biotehnološkega načina produkcije z ursolno kislino (UA) obogatenega rastlinskega izvlečka žajblja (*Salvia officinalis* L.), smo uvedli *in vitro* kulturo poganjkov žajblja dveh akcesij (HAM13 - SALV 25/89 ter DB 13/6). Določili smo optimalne pogoje za ekstrakcijo UA (temperatura sušenja biomase, ekstrakcijsko topilo, čas ekstrakcije). Optimirali smo sestavo gojišča za mikropropagacijo poganjkov in za indukcijo kalusnega tkiva. Ugotovili smo razliko v sposobnosti akumulacije UAmed celičnimi kulturami induciranimi iz baze poganjka, lista, peclja in korenine. Selekcionirali smo dve morfološki obliki kalusnega tkiva (drobljiv in kompakten), ki sta pokazali različno hitrost rasti in sposobnosti akumulacije UA. Določili smo kinetiko rasti in sinteze UA v obeh morfoloških oblikah. Spremljali smo kinetiko razvoja agregirane celične kulture iz enocelične suspenzije in vpliv velikosti delcev na vsebnost UA. V celični suspenzijski kulturi smo preizkusili vpliv svetlobe, dodatka različnih rastnih regulatorjev (kinetin, BA, NAA, 2,4-D, IAA, GA3), vpliv koncentracije saharoze (0, 30, 60 g/l), mehanskega stresa (hitrost stresanja, gostota, ultrazvok), vpliv prekurzorja (skvalen), vpliv elicitorjev (ABA, SA, JA, MeJ, hitin) na rast in akumulacijo UA. Preizkusili smo vpliv dodatka antibiotika (penicilin G, streptomicin) in antimikotika (amfotericin B) na rast biomase in akumulacijo UA. Potrdili smo protivnetno aktivnost izvlečka biotehnološko pridelane biomase. Določili smo kinetiko rasti, produkcije UA in vitalnost celične kulture na stresalniku in jo primerjali z isto biomaso kultivirano v bioreaktorju. Spremljali smo potek rasti posamezne velikostne frakcije delcev biomase (premer delca: d_p <100 µm <380 µm <1100 µm < d_p) v kulturi na stresalniku in v bioreaktorju. Primerjali smo produktivnost biotehnološkega procesa proizvodnje UA s konvencionalno poljsko pridelavo.

Optimisation of Ursolic Acid Production from Sage (*Salvia officinalis* L.) in Bioreactor

Ž. Bolta
Biotechnical Faculty, University of Ljubljana, Slovenia
Supervisor: D. Baričevič
Biotechnical Faculty, University of Ljubljana, Slovenia

Biotechnological production potential of an enriched sage (*Salvia officinalis*) extract has been evaluated using an *in vitro* shoot culture, callus culture and cell suspension culture induction from two sage accessions (HAM13 - SALV 25/89 and DB 13/6). For further investigation the accession with the highest ability of UA accumulation was chosen optimal extraction conditions were set (biomass drying time and temperature, extraction solvent, extraction time). The medium for micropropagation, callus and root tissue culture induction was optimised for high growth rate. The callus and suspension cultures of different plant organs (apex, leaf, stem, root) were studied. Differences in growth and UA accumulation kinetics were found between different morphological forms of callus tissue. The aggregate growth form a single cell culture was followed and a strong influence of particle size distribution on UA accumulation was found out. In cell suspension culture the influence of illumination, plant growth regulators (Kinetin, BA, NAA, 2,4D, IAA, GA), sucrose concentration (0, 30, 60 g/l), mechanical stress (rotation speed, culture density, ultrasound), precursor (squalene), elicitors (ABA, SA, JA, MeJ, chitin), antibiotic (penicillin G, streptomycin) and antimicotic (amphotericin B) was investigated on growth and UA accumulation. The anti-inflammatory activity of extract from biotechnologically produced biomass was confirmed with mice ear edema test. The growth, viability and UA accumulation and particle size distribution (d_p <100µm <380µm <1100µm <d_p) of cell culture form bioreactor and shake flasks were compared. The UA productivity in bioreactor was compared to the conventional, field, production.

Razvoj metode izolacije inhibitorjev trombina iz *Stereum hirsutum*

K. Borštar
Fakulteta za farmacijo, Univerza v Ljubljani, Slovenija
Mentor: S. Kreft
Fakulteta za farmacijo, Univerza v Ljubljani, Slovenija

Bolezni srca in ožilja so zelo pogosta obolenja in predstavljajo enega najpogostejših vzrokov smrti sodobnega človeka. Trombin ima kot serinska proteaza pri tem ključno vlogo, saj sodeluje pri procesih strjevanja krvi in razvoju tromboze. Zaradi tega predstavlja primerno tarčo za inhibicijo.
Rešetanje nekaterih izbranih vrst gliv glede inhibitorne aktivnosti na trombin je pokazalo, da dlakava slojevka (*Stereum hirsutum*) zmanjšuje aktivnost trombina. Na osnovi teh rezultatov smo se odločili razviti metodo izolacije aktivne komponente iz glivnega materiala.
Metanolni izvleček dlakave slojevke smo pripravili iz 1 kg droge in ga macerirali v 6 L metanola. Po odparitvi topila smo suh izvleček raztopili v ustrezni mobilni fazi in ga uporabili za kolonsko in tankoplastno kromatografijo. Po razvijanju vzorca v sedmih mobilnih fazah, v različnih razmerjih diklormetana in metanola, smo se odločili za mobilno fazo diklormetan:metanol 5:1. Inhibitorno aktivnost trombina smo spremljali s pomočjo spektrofotometra na mikrotitrskih ploščicah, na katerih smo merili absorbanco produkta, ki nastaja po amidolitični cepitvi substrata S-2238.
Med razvojem metode za izolacijo smo spremljali inhibitorno aktivnost posamezni frakciji in nadaljevali z najbolj aktivno frakcijo. Rezultati meritve inhibicije na trombin kažejo ponovljive inhibitorne aktivnosti. Pri zadnjih dveh kolonskih kromatografijah smo uporabili mobilno fazo diklormetan - metanol 20:1, da bi dobili bolj selektivno ločene lise, a smo ugotovili, da imamo še vedno prisotnih veliko lis, ki v prejšnji mobilni fazi niso bile vidne. Dobro bi bilo poskusiti še druge kromatografske tehnike, ki bi dale več rezultatov o fizikalno-kemijskih lastnostih aktivne spojine.

Development of Isolation Method for Thrombin Inhibitors from *Stereum hirsutum*

K. Borštar
Faculty of Pharmacy, University of Ljubljana, Slovenia
Supervisior: S. Kreft
Faculty of Pharmacy, University of Ljubljana, Slovenia

Cardiovascular diseases are very often disorders that represent one of the most frequent cause of death in the modern world. Thrombin, a serin proteinase, plays a key role in the process of blood coagulation and in the development of thrombosis and consequently represents a proper target for inhibition.
Screening of selected types of fungi for their inhibitory activity on thrombin showed that *Stereum hirsutum* has inhibitory activity on thrombin. On the basis of these results, a decision was taken to develop a method for isolation of the active component from fungal material.
The methanol extract of *Stereum hirsutum* was prepared from 1 kg of drugs and macerated in 6 L of methanol. After evaporation of the solvent, the dry extract was dissolved in a suitable mobile phase and then used for the column and thin-layer chromatography. After developing the sample in seven mobile phases in different dichloromethane and methanol proportions, the mobile phase dichloromethane - methanol =5:1 was selected. The inhibitory activity of thrombin was monitored with a spectrophotometer on microtiter plates, on which the absorbance was measured after the amidolytic breakdown of the substrate S-2238.
During the development of the isolation method we followed the inhibitory activity of the fractions and proceeded with the most active one. The results of the measured inhibition show steady inhibitory activities. In the last two column chromatographies, the mobile phase dichloromethane : methanol = 20:1 was used to obtain more selectively separated spots. However, a lot of spots that were not seen in the previous mobile phase were found to be still present in this mobile phase.

Oglaševalsko komuniciranje preko medijev

D. Car
Visoka šola za upravljanje in poslovanje, Novo mesto, Slovenija
Mentor: M. Blažič
Visoka šola za upravljanje in poslovanje, Novo mesto, Slovenija

Tržna komunikacija je proces, pri katerem oglaševalci, bodisi podjetja bodisi oglaševalske agencije, pošiljajo sporočila preko medijev ali osebne prodaje o izdelkih svojim ciljnim skupinam. Je specifičen način komunikacije, saj je potrebno preko vizualnega izgleda izdelka povedati vse pomembne stvari ter s tem prepričati kupce o nakupu. Zajema pojme, kot so oglaševanje, pospeševanje prodaje, stiki z javnostjo, osebna prodaja. Pomemben prenosnik sporočila so mediji, ki prenesejo sporočilo od pošiljatelja k recipientu oziroma sprejemniku. Vsak medij je zase specifičen, zato so tudi oglaševalska sporočila, ki jih posredujejo, prilagojena vsakemu mediju posebej. Tržna komunikacija se razvija v neznano smer, jasno je le, da bodo ustaljeni trženjski prijemi postali preslabi za prepričanje o nakupu.
Naloga prikazuje primer tržne komunikacije v podjetju Krka, tovarna zdravil oziroma trženje osebne blagovne znamke My LR Beauty. Z metodo osebnega anketiranja, ki je bila izvedena na področju Novega mesta in širše okolice, smo skušali ugotoviti stopnjo poznavanja blagovne znamke, po katerih informacijskih kanalih oziroma prenosnikih sporočila se je kupec seznanil z znamko ter s tem posredno določiti najučinkovitejši način tržne komunikacije za tovrstno blagovno znamko.
Ker gre za kozmetičen proizvod, proizvajalci in predvsem pospeševalci prodaje dajejo zelo velik poudarek na vizualne in funkcionalne prednosti izdelka. Rezultati so pokazali, da se kupci tovrstnih blagovnih znamk odločajo prav na podlagi vizualne strani izdelka, nič manj pomena pa ne dajejo njegovi uporabnosti in učinkovitosti.
Kot končni in nedvomno zadovoljivi rezultat se je pokazalo, da je znamka My LR Beauty v močni konkurenci dobro poznana in zadovoljiva znamka, za katero se kupci radi odločajo.

Marketing Communication through Media

D. Car
School of Business and Management, Novo mesto, Slovenia
Supervisor: M. Blažič
School of Business and Management, Novo mesto, Slovenia

Marketing communication is a process in which the advertisers, either the companies or advertising agencies send messages and information on products through media or personal sale. It is a specific mode of communication because through visual appearance of the product it is necessary to tell all important things and persuade the buyers for purchase. It includes terms, such as advertising, sales promotion, public relations, personal sale… Media are sending important messages to the recipient or receiver. Each advertising media is specific and so are the advertising messages, which they are sending, adjusted to each media separately. Marketing communication is being developed to an unknown direction. It is just clear that steady marketing techniques became too weak to persuade of purchase.
The theme is presenting an example of market communication in the company Krka, tovarna zdravil, i.e. marketing of its trademark My LR Beauty. Using a personal questionnaire method, conducted in Novo mesto area and far vicinity we tried to find out level of knowledge of trademark, through which channels information and/or information system the buyer has informed himself on trademark, and that indirectly determine or establish the most effective model of market communication for such a trademark.
As it goes for cosmetic product, manufacturers and, first of all promoters of sale are laying great emphasis on visual and functional advantages of the product. Result showed that the buyers of such trademark made up their mind just on the basis of visual side of the product, but not less significance they give to its use and efficacy.
As final and undoubtedly satisfactory result it turned out that the trademark My LR Beauty is in keen competition well known and satisfactory trademark and the buyers are willing to decide for it.

Pomen računovodskih informacij za doseganje strateških ciljev slovenskih podjetij

S. Čadež
Ekonomska fakulteta, Univerza v Ljubljani, Slovenija
Mentor: M. Hočevar
Ekonomska fakulteta, Univerza v Ljubljani, Slovenija

Strateško računovodstvo za razliko od tradicionalnega poslovodnega računovodstva pripravlja informacije za najvišji management. Usmerjeno je navzven iz podjetja, poudarek daje prihodnosti in je osredotočeno na dolgi rok. Pojem strateško računovodstvo se je prvič pojavil leta 1981 in tudi po dveh desetletjih je glavni izziv računovodij pripraviti informacije na tak način, da jih lahko managerji uporabijo pri strateških odločitvah.
Strateško računovodstvo se ukvarja s poslovnimi strategijami, ki določajo konkurenčni položaj znotraj panoge. Namen poslovne strategije je prodati določeno skupino proizvodov določeni skupini kupcev in biti pri tem uspešnejši od konkurentov. Celoten proces strateškega managementa lahko razdelimo na štiri stopnje: analiza okolja, razvoj strategije, uresničevanje strategije in strateška kontrola. V vsaki stopnji so informacijske potrebe drugačne in specifične.
Naloga skuša združiti posamezne tehnike in orodja strateškega računovodstva v teoretični okvir in v drugem delu na podlagi empiričnih podatkov oceniti dejansko uporabo in koristnost teh tehnik v slovenskih podjetjih. Glavne ugotovitve raziskovalne naloge so tri: (1) mnoga slovenska podjetja že uporabljajo posamezne tehnike strateškega poslovodnega računovodstva, (2) uporaba računovodskih informacij pri odločanju pozitivno vpliva na uspešnost in (3) kljub koristnosti računovodskih informacij managerji le-te pri sprejemanju odločitev premalo upoštevajo. Če povzamem v enem stavku: računovodstvo za notranje uporabnike je koristno, saj uporaba računovodskih informacij pri odločanju vodi do večje uspešnosti podjetij.

The Role of Accounting Information in Attainment of Strategic Goals of Slovenian Companies

S. Čadež
Faculty of Economics, University of Ljubljana, Slovenia
Supervisor: M. Hočevar
Faculty of Economics, University of Ljubljana, Slovenia

Strategic management accounting (SMA) is oriented to the needs of senior management. SMA has a characteristic external focus and is oriented towards the (long-term) future. The term strategic management accounting first appeared 20 years ago. Two decades later, the major contemporary challenge of accountants is to provide the relevant information configured in a way in which it can be used for strategy.
SMA deals with business strategies. The aim of a business strategy is to sell a distinct product group to a distinct group of customers more effectively than the competitors. The process of strategic management can be divided into four stages: environmental scanning, strategy formulation, strategy implementation and strategic control. Obviously, information needs differ in each stage and are served by various techniques that comprise the framework of strategic management accounting.
This paper aims to synthesise existing SMA techniques, advocated by various authors, into a theoretical framework and to appraise actual usage and usefulness of these techniques in large Slovenian companies. Three major findings can be distilled from this study. The analysis of survey data shows (1) that many Slovenian companies make use of individual tools of SMA and (2) that usage of strategic accounting information appears to be positively related to company performance. (3) Last finding relates to managerial behaviour in Slovenia. The study indicates that despite its apparent usefulness managers don't sufficiently exploit provided strategic information. To summarize briefly, accounting for internal users is beneficial and leads to better performance.

Vzpostavitev procesne organizacije z uporabo objektno usmerjenenega modeliranja in "Rapid Re" metodologije

A. Dobrovoljc
Fakulteta za računalništvo in informatiko, Univerza v Ljubljani, Slovenija
Mentor: M. Mihelčič
Fakulteta za računalništvo in informatiko, Univerza v Ljubljani, Slovenija

Tekma za kupce, ki zahtevajo proizvode, prilagojene njihovim posebnim željam in potrebam, narekuje proizvajalnim podjetjem visoko raven učinkovitosti, prilagodljivosti in inovativnosti. Tem zahtevam mora slediti organizacija podjetja, ki močno vpliva na njegovo poslovno uspešnost. Poleg navpične, hierarhično zasnovane organizacije potrebujemo tudi vodoravno, procesno usmerjeno organizacijo, ki podpira delovni proces preko meja posamičnih poslovnih funkcij. Uveljavitev bolj procesne organizacije je običajno povezana s temeljitim preustvarjanjem poslovnih procesov (PPP). Zaradi uvajanja tako tehničnih kot tudi družbenih sprememb v združbi sodijo projekti PPP med tiste z visokim tveganjem. Možnosti za uspeh si lahko povečamo z uporabo ustreznih orodij in primernih metodologij. V nalogi sem proučil metodologijo hitrega preustvarjanja poslovnih procesov "Rapid Re", oblikoval konkretnemu podjetju prilagojen projekt PPP in ga preizkusil v praksi. Metodologijo sem izboljšal z uporabo objektno usmerjenega modeliranja. Zaradi lažjega izbora ali izgradnje ustreznega modela informacijskega sistema na osnovi ustvarjenega poslovnega modela sem uporabil poenoten jezik za modeliranje UML. Ob uresničevanju projekta v praksi sem posamezne stopnje in korake metodologije, ki vključuje ustrezne informacijske prvine, preveril tudi z drugimi uveljavljenimi pristopi in orodji ter s tem poizkušal povečati zanesljivost uresničevanja. V drugem delu naloge predstavljam ugotovitve in dosežke izvedenih stopenj projekta s poudarkom na oblikovanju rešitve ter načrtovanju vzpostavitve bolj procesne organizacije.

The Implementation of a Process-oriented Organization through Object Oriented Modelling and "Rapid Re" Methodology

A. Dobrovoljc
Faculty of Computer and Information Science, University of Ljubljana, Slovenia
Supervisor: M. Mihelčič
Faculty of Computer and Information Science, University of Ljubljana, Slovenia

A competition for customers, who require products that fit their special needs and expectations, dictates producers to maintain a high level of efficiency, flexibility and innovativeness. In order to stay competitive, an enterprise organization has to be based on these criteria. Besides vertical, hierarchically structured organization there has to be a horizontal, work-process oriented organization that supports a work process beyond the functional levels of the company. The implementation of a more work-process oriented organization usually requires a complete business process re-engineering (BPR). Because of the introduction of both technical and social changes into the company, BPR projects are considered to be extremely risky. However, a possibility for a successful implementation can be improved by using the right tools and the appropriate methodologies. In the thesis I presented the methodology of a rapid business process re-engineering "Rapid Re", designed a BPR for a particular enterprise and proved its effectiveness in a real-life environment. I improved the methodology by using object oriented modelling. In order to be able to choose or create the appropriate information system that would fit the existing business model, I decided to use unified modelling language UML. While putting the project into practice I checked each stage and step of the methodology, which includes the corresponding information resources, with other proved approaches and tools and thus tried to increase the reliability of its implementation. In the second part of the thesis I present my findings and the results of the project stages carried out, while proposing a solution and a plan to create a more process-oriented organization.

Norfloxacine - Primary and Secondary Prophylaxis of Spontaneous Bacterial Peritonitis in Patients with Cirrhosis and Ascites

A. Donkova - Marinova
Medical Faculty, Sofia, Bulgaria
Supervisor: K. G. Tchernev
Medical Faculty, Sofia, Bulgaria

Background: Spontaneous bacterial peritonitis (SBP) is considered a bacterial infection of ascitic fluid without any intraabdominal, surgically treatable source of infection. Cirrhotic patients with ascites and low ascitic fluid total protein levels are at high risk to develop the first episode of SBP during long-term follow up.
Aims: The aim of this study is to determine the efficacy of long-term administration of norfloxacin in the primary and secondary prevention of SBP in high risk patients.
Methods: Fifty -three consecutively hospitalized cirrhotic patients with ascites during an 18 months period were included in this prospective study. All underwent a diagnostic paracenthesis with determination of ascitic total protein and albumin, white blood cell count (WBC). 24 patients were found to have ascitic total protein below 15g/l and 4 patients had WBC > 500/ml in the ascitic fluid. The 24 patients with low ascitic protein were randomized in two groups: Group I (12 patients) received 400 mg/d PO norfloxacin. Group II (12 patients) received no treatment. The patients in groups I and II had no previous SBP. The patients with SBP (Group III) were initially treated with ceftriaxon 1g/12 h IV for 5 days and after the resolution of the symptoms (WBC< 250/l) were given 400 mg/d of norfloxacin. All patients were treated and followed up for 6 months. There were no significant differences in age, sex and etiology of the cirrhosis among the patients in the three groups.
Results: During the follow up there was no SBP in group I (0/12), 2 (2/12) SBPs developed in group II and 1 (1/4) in group III. One of the patients in group III died of variceal hemorrhage. The incidence of extra peritoneal infections were similar in groups I and II -3/12 urinary tract infections. No extraperitoneal infections were observed in group III. No resistance to norfloxacin was observed in the isolated *E. coli* in the patients from group I, although treatment with norfloxacin induced development of norfloxacin-resistant gram-negative bacteria in 5 of the treated patients (5/16) within mean time of 18,5+/- days after the initiation of treatment.
Conclusions: Continuous long-term prophylaxis with norfloxacin is effective in preventing the first spontaneous bacterial peritonitis in cirrhotic patients at high risk and reduces the risk of a second episode of SBP. The development of norfloxacin resistant flora does not seem to have clinical significance in our patient population that is not previously treated with quinolone antibiotics.

Tkivno-specifično uravnavanje izražanja holesterogenih genov

K. Fon Tacer
Medicinska fakulteta, Univerza v Ljubljani, Slovenija
Mentorica: D. Rozman
Medicinska fakulteta, Univerza v Ljubljani, Slovenija

Preučili smo izražanje genov, vključenih v biosintezo holesterola, v modih podganjih in mišjih samcev. Količina mRNA genov, ki nosijo zapis za encime, odgovorne za nastanek MAS (mejozo aktivirajočih sterolov), tekom spolnega razvoja podganjih samcev narašča, medtem ko se količina genov, ki kodirajo encime za pretvorbo MAS do holesterola, ne spremeni oz. celo pade. Predpostavljamo, da je neskloplјeno uravnavanje izražanja genov biosintetske poti holesterola prvi mehanizem, ki vodi v kopičenje po-skvalnega intermediata- T-MAS v modih. Da bi ugotovili vlogo od cAMP-odvisnega transkripcijskega faktorja CREM pri uravnavanju biosintetske poti holesterola v modih, smo naredili eksperimente na DNA mikromrežah, ki so vsebovale izbrane gene, vključene v procese homeostaze holesterola. Izražanje CYP51 je v modih miši z izničenim genom CREM po pričakovanjih znižano. Nepričakovano pa se izražanje sterol C4-metil oksidaze, prvega encima v pretvorbi T-MAS proti holesterolu, ob odsotnosti gena CREM poveča. To je do sedaj prvo odkritje povečanja izražanja kot posledica izničenja gena CREM. V človeški celični liniji horionskih resic JEG-3 smo z znižanjem ravni transkripcijskih faktorjev SREBP želeli simulirati pogoje, pod katerimi poteka biosinteza holesterola v modih. CYP51 in sintezo holesterola pa smo aktivirali preko od cAMP-odvisne signalne poti. Simulacija ni popolno uspela, saj smo ob aktivaciji cAMP poti, ki je v modih odgovorna za sintezo T-MAS, opazili padec količine vseh merjenih intermediatov. Po drugi strani pa smo opazili zmanjšanje koncentracije holesterola, kar je v skladu z metabolizmom moških spolnih celic, ki slabo proizvajajo holesterol *de novo*. Prepisovanje gena CYP51, ki se je ob sterolni represiji znižalo za 50%, se je ob stimulaciji s cAMP ponovno zvišalo do začetne ravni. S poskusi na mikromrežah smo ugotovili, da celična linija JEG-3 ni najprimernejši modelni sistem za preučevanje izražanja genov, vključenih v homeostazo holesterola.

Tissue-specific Regulation of Cholesterogenic Genes Expression

K. Fon Tacer
Faculty of Medicine, University of Ljubljana, Slovenia
Supervisor: D. Rozman
Faculty of Medicine, University of Ljubljana, Slovenia

We investigated the expression of cholesterogenic genes in mouse and rat testis. Expression of genes, encoding enzymes involved in MAS (meiosis activating sterols) synthesis, is upregulated during sexual development of the rat. Expression level of genes, responsible for MAS conversion toward cholesterol, is low and even decreases during maturation of the animal. We propose that the lack of coordinate transcriptional control over cholesterol biosynthetic pathway is the first mechanism leading to T-MAS accumulation in testis. To evaluate the role of cAMP-dependant transcription factor CREM in regulation of cholesterol biosynthetic pathway, we performed experiments on DNA microarray containing selected genes involved in cholesterol homeostasis. As expected, the expression of CYP51 is down-regulated in testis of CREM-knockout mice, but it was very unexpected to find sterol C4-methyl oxidase (the first enzyme converting T-MAS toward cholesterol) upregulated in CREM-knockout animals. This is the first observation of gene expression upregulation as a consequence of CREM deficiency. In order to simulate the conditions of cholesterol biosynthesis in testis, we reduced the level of transcription factors SREBP in human choriocarcinoma cell line JEG-3. Activation of CYP51 and cholesterol biosynthesis was triggered through cAMP-dependent pathway. Simulation was not completely successful, since we noticed a decreased amount of all analysed sterol intermediates after stimulating the cAMP pathway that is in testis responsible for T-MAS accumulation. On the other hand, the decrease of cholesterol quantity was noticed, which is in accordance with metabolism of male germ cells that are not efficient in producing cholesterol *de novo*. mRNA level of CYP51 was reduced for 50% by sterol repression and increased by stimulation of cAMP pathway to the initial level.

Ovrednotenje vloge in pomena fitoterapije ter analiza informacij o interakcijah fitoterapevtikov v spremnih listih

J. Godnov
Fakulteta za farmacijo, Ljubljana, Slovenija
Mentor: M. Kos
Somentor: V. Kmetec
Fakulteta za farmacijo, Ljubljana, Slovenija

V diplomski nalogi so navedene informacije o fitoterapiji in fitoterapevtikih, izpostavitev temeljnih problemov, ki se pojavljajo v zvezi s takimi zdravili, opredelitev vloge fitoterapije v družbenem okolju in ocena možnih perspektiv tega področja v okviru farmacevtske skrbi.
Izbrali smo kvalitativno metodo, polstrukturiran razgovor, kjer so nam zdravniki, farmacevti, zeliščar in laična javnost podali svoj vidik in opredelili svoje stališče do fitoterapije. Združili smo odgovore glede na posamezna vprašanja, ki so zajemala odnos do fitoterapetikov, varnost in učinkovitost fitoterapevtikov in vlogo farmacevta, zdravnika in zeliščarja pri zdravljenju s fitoterapevtiki, ter jih komentirali.
Po ključu največje uporabe in prodaje zdravilnih rastlin v Sloveniji in po svetu smo pregledali spremne liste registriranih fitoterapevtikov v Sloveniji, ki vsebujejo šentjanževko, ginseng, glog, česen, pegasti badelj in baldrijan, po mnenju stroke pa smo analizirali tudi pripravke s seno in cimicifugo. Obenem smo pregledali tudi nekatere spremne liste sinteznih zdravil (najpogosteje predpisane učinkovine leta 2000; enalapril, simvastatin in amoksicilin s klavulansko kislino, vključili smo tudi učinkovini zolpidem in varfarin). Glede na vključenost in obširnost informacij o interakcijah smo postavili oceno.

Evaluation of the Role and Importance of Phytotherapy and Analysis of Information of Herb-Drug Interactions in Patient Information Leaflets

J. Godnov
Faculty of Pharmacy, Ljubljana, Slovenia
Supervisor: M. Kos
Co-supervisor: V. Kmetec
Faculty of Pharmacy, Ljubljana, Slovenia

In my diploma thesis, information of phytotherapy and phytotherapeutics, exposition of basic problems occurred with herbal medicines, definition of phytotherapy`s role in social environment and evaluation of perspectives in phytotherapy concerning pharmaceutical care are stated.
We chose qualitative method, half-structured interview, where doctors, pharmacists, herbalist and laic publicity expressed their views and defined their attitude towards phytotherapy. We exposed the main thoughts of all interviewees and sorted them in categories; one of these categories was taken from phytotherapeutics`s safety, herb-drug interactions.
The main source of information about phytotherapeutics`s safety is patient information leaflet. We verified the patient information leaflets of phythotherapeutics registered in Slovenia which contained St. John`s wort, Ginseng, Hawthorn, Garlic, Milk Thistle and Valerian according to the largest usage. We also analysed preparations with Senna and Black Cohosh. We also verified some patient information leaflets of synthetic medications (non-herbal medicines) - the most frequently prescribed substances in year 2000 - enalapril, simvastatin and amoxicilin with clavulanic acid. We included zolpidem and warfarin substances too. According to inclusions and comprehension of interaction's information we set the values. The final analysis showed that despite well documented and introduced interactions of some herbs there are poorly described and listed interactions in patient information leaflets of phytotherapeutics and synthetic medicines.

Spremljanje rasti biomase pri šaržnem fermentacijskem procesu

B. Gregorčič
Fakulteta za strojništvo, Univerza v Ljubljani, Slovenija
Mentor: I. Golobič
Fakulteta za strojništvo, Univerza v Ljubljani, Slovenija

V fermentacijskem procesu s končnim produktom v obliki primarnih ali sekundarnih metabolitov je ena izmed zelo pomembnih veličin biomasa, ki karakterizira kvaliteto fermentacijskega procesa. Rast biomase smo spremljali s pomočjo merjenja koncentracij kisika in ogljikovega dioksida v odduhu iz fermentorja. Meritve smo izvedli na 80 m^3 fermentorjih v Krki, d. d., Novo mesto. Z ocenjevalnim modelom, ki temelji na metodi najmanjših kvadratov s časovno spremenljivim faktorjem pozabljanja, smo predstavili možnost sprotnega spremljanja specifične hitrosti rasti biomase, iz česar smo določili koncentracijo biomase in vsebnost končnega produkta pri različnih šaržah fermentacije bacitracina in pri fermentaciji salinomicina.
Zaključimo lahko, da je sprotno ocenjevanje rasti biomase pomembno za oceno kvalitete fermentacijskega procesa. Z merjenjem koncentracije kisika in ogljikovega dioksida imamo na voljo dva neodvisna ocenjevalna algoritma, s katerima je možno analizirati različne aerobne fermentacijske procese. Primerjava ocenjenih z izmerjenimi vrednostmi biomase in vsebnosti končnega produkta potrjuje obetavnost ocenjevalnega modela.

Monitoring of Biomass Growth Rate at Batch Fermentation Process

B. Gregorčič
Faculty of Mechanical Engineering, University of Ljubljana, Slovenia
Supervisor: I. Golobič
Faculty of Mechanical Engineering, University of Ljubljana, Slovenia

In fermentation process with final product in the form of primary or secondary metabolites biomass is an essential parameter that characterizes the quality of fermentation process. Biomass growth rate has been determined based on measurements of oxygen and carbon dioxide concentration in exhaust gas of bioreactor. Measurements have been performed on 80 m^3 bioreactors in Krka, d. d., Novo mesto. With estimative model, based on least squares method with time-varying forgetting factor, we have presented the feasibility of on-line monitoring of biomass specific growth rate. On that basis we have determined biomass concentration and final product content at different batches of bacitracin fermentation and fermentation of salinomicin.
We can conclude that on-line monitoring of biomass growth rate is important for evaluation of fermentation process quality. Measurements of oxygen and carbon dioxide concentration give us two independent estimating algorithms, which are able to analyse different aerobic fermentation processes. Comparison of estimated with measured values of biomass concentration and final product content looks promising for evaluating model.

Kloniranje in določitev nukleotidnega zaporedja odseka DNA za proteolizno aktivnost bakterije *Streptomyces rimosus*

K. Grlica
Krka, d. d., Novo mesto, Slovenija
Mentorica: I. Mandič-Mulec
Biotehniška fakulteta, Ljubljana, Slovenija
Somentor: A. Gasparič
Krka, d. d., Novo mesto, Slovenija

Bakterijske proteaze so industrijsko pomembni encimi. Med stacionarno fazo bakterija *Streptomyces rimosus* proizvaja oksitetraciklin in tudi proteolitične encime.
Da bi proučevali proteolitične encime bakterije *S. rimosus*, smo fragmente njene kromosomske DNA klonirali v plazmid pIJ699 gostiteljske bakterije *Streptomyces lividans* 1326. V tem gostitelju smo sledili spremembo proteolitične aktivnosti klonov, ki so nosili rekombinantne plazmide. Dva klona *S. lividans* (št. 7 in št. 29), ki sta nosila rekombinantna plazmida pSI7 in pSI29, sta na mlečnem agarju pokazala povečano proteolitično aktivnost v primerjavi s kontrolnim sevom, ki je nosil le plazmid pIJ699 brez kromosomske DNA bakterije *S. rimosus*. Fragmente genomske DNA bakterije *S. rimosus* smo izolirali iz plazmida pSI7 rezanega s HindIII, jih subklonirali v plazmid pUC19 in z rekombinantnim plazmidom transformirali bakterijo *E. coli*. Plazmide smo izolirali in jih analizirali z restrikcijskimi encimi. Trem plazmidom, ki so nosili genomsko DNA bakterije *S. rimosus*, smo določili nukleotidno zaporedje. Računalniška analiza nukleotidnih zaporedij je pokazala, da sta imeli dve zaporedji (7H2r in 7H9u) homologijo z zaporedjem, ki kodira hipotetično feredoksin/feredoksin NADP+ reduktazo bakterije *Streptomyces coelicolor* A3(2). Obe zaporedji sta se s homolognim zaporedjem ujemali v 44% aminokislin. Zaporedju 7H3r pa smo našli homologijo z zaporedjem za hipotetični regulator (ujemanje aminokislin je 30 - odstotno) in z zaporedjem za hipotetični protein (29 - odstotno ujemanje aminokislin) bakterije *S. coelicolor* A3(2).

Cloning and Sequencing of *Streptomyces rimosus* DNA Fragment for Proteolitic Activity

K. Grlica
Krka, d. d., Novo mesto, Slovenia
Supervisor: I. Mandič-Mulec
Biotechnical Faculty, University of Ljubljana, Slovenia
Co-supervisor: A. Gasparič
Krka, d. d., Novo mesto, Slovenia

Bacterial proteases have a great industrial value. During stationary growth *Streptomyces rimosus* produces oxytetracycline and also synthesizes proteolytic enzymes. In order to study proteolytic enzymes of these bacteria we cloned genomic DNA fragments of *Streptomyces rimosus* into the plasmid pIJ699 of an "assistant" host, *Streptomyces lividans* 1326. We followed protease activity of *S. lividans* clones, which carried recombinant plasmids. Two clones (number 7 and number 29), which carried pSI7 and pSI29 plasmids had shown increased protease activity on milk agar plates in comparison with *S. lividans* carrying only the control plasmid, pIJ699. We isolated genomic DNA fragments from pSI7 plasmid cut with HindIII and subcloned them into pUC19 of *E. coli*. We isolated three different plasmids which carried *S. rimosus* genomic DNA from pSI7 in the pUC19 plasmid. We computer analyzed the cloned fragments and found that sequences 7H2r and 7H9u were 44% identical to the amino acid sequence of the putative ferredoxin/ferredoxin NADP+ reductase of *S. coelicolor* A3(2). The 7H3r sequence showed similarity with the amino acid sequences of a putative regulator (30% identical amino acids) and with the sequence of a hypothetical protein (29% identical aminoacids) from *S. coelicolor* A3(2).

Raziskave kompleksa stefina A in papaina s pomočjo jedrske magnetne resonance

I. Hafner
Fakulteta za kemijo in kemijsko tehnologijo, Univerza v Ljubljani, Slovenija
Mentor: R. Jerala
Kemijski inštitut, Ljubljana, Slovenija

S pomočjo jedrske magnetne resonance (NMR) smo raziskovali vpliv tvorbe kompleksa s papainom na strukturo inhibitorja stefina A. Molekulska masa kompleksa stefin A - cisteinska proteaza papain je 34 kDa, kar je na meji zmogljivosti današnjih tehnik NMR. Izotopska obogatitev samo enega od proteinov v kompleksu (stefina A) nam je omogočila uporabo večdimenzionalnih heteronuklearnih eksperimentov NMR, s katerimi smo opazovali le signale inhibitorja.
Pripravili in izolirali smo 20 mg rekombinantnega proteina stefina A, označenega s stabilnim izotopom ^{15}N. Visoko topnost kompleksa, ki je potrebna za eksperimente NMR, smo dosegli z dodatkom DMSO (do 10%) pri pH 6 ali z znižanjem pH na 4.3. S fluorimetričnimi meritvami smo dokazali, da se pri omenjenih pogojih kinetične in ravnotežne konstante interakcije stefina A s papainom bistveno ne razlikujejo od tistih pri pH 6. Posneli smo dvodimenzionalne (2D) 1H-15N HSQC spektre prostega stefina A ter 2D ^{1}H-^{15}N HSQC in 3D ^{1}H-^{15}N NOESY-HSQC spektre kompleksa izotopsko obogatenega stefina A s papainom. Opazovali smo spremembe kemijskih premikov signalov ogrodnih amidnih skupin inhibitorja, do katerih pride zaradi tvorbe kompleksa. Znatne spremembe kemijskega okolja smo tako opazili pri manj kot tretjini aminokislinskih ostankov, in sicer na N-terminalnem koncu (I7 - G10), v prvi (Q53 - G57) ter v drugi vezavni zanki (S102 - D107).
Ugotovili smo, da se N-terminalni ostanki (I7 - G10), ki so v prostem inhibitorju neurejeni, vežejo na encim, prvi aminokislinski ostanek M6 pa kljub tvorbi kompleksa ostane relativno gibljiv in malo prispeva k interakciji. To je v skladu s kinetičnimi študijami mutant stefina A iz literature, medtem ko je v kristalni strukturi kompleksa homolognega proteina stefina B s papainom M6 dobro definiran.

NMR Study of Stefin A - Papain Complex

I. Hafner
Faculty of Chemistry and Chemical Technology, University of Ljubljana, Slovenia
Supervisor: R. Jerala
National Institute of Chemistry, Ljubljana , Slovenia

We have investigated changes in stefin A structure upon formation of complex with cisteine protease papain by nuclear magnetic resonance (NMR). Size limitation is the largest limitation of NMR in structural biology, so the stefin A - papain complex (34 kDa) represents a challenge for modern solution NMR. Isotopic enrichment of one partner in the complex (stefin A) enabled us to perform multidimensional experiments in the way that only inhibitor signals were observed.
We prepared and isolated 20 mg of recombinant ^{15}N-labeled stefin A. The high solubility of complex needed for NMR experiments was accomplished either by addition of DMSO (up to 10%) at pH 6 or by decreasing pH to 4.3. Both ways fluorimetrically measured kinetic and equilibrium constants of interaction between stefin A and papain did not change significantly in comparison to those at pH 6. 2D ^{1}H-^{15}N HSQC spectra of free stefin A, 2D ^{1}H-^{15}N HSQC and 3D ^{1}H-^{15}N NOESY-HSQC spectra of labeled stefin A - unlabeled papain complex were recorded. We investigated the change in chemical shifts of inhibitor's backbone amide groups upon complex formation. Less than one third of backbone amide groups experienced considerable chemical shift change, namely those of the residues in the N-terminal trunk (I7 - G10), the first (Q53 - G57) and the second binding loop (S102 - D107).
Our results show that N-terminal residues I7 - G10, which are flexible in free inhibitor, bind to enzyme, but M6 remains flexible and does not participate extensively in complex formation. This is in accordance with kinetic studies of stefin A mutants reported in the literature, while M6 seems to be well defined in X-ray crystal structure of the complex of homologous protein stefin B and papain.

Analiza mutacij v popravljalnem genu *hMLH1* pri slovenskih bolnikih z rakom želodca

P. Hudler
Medicinska fakulteta, Univerza v Ljubljani, Slovenija
Mentor: S. Repše
Univerzitetni klinični center Ljubljana, Slovenija
Somentor: R. Komel
Medicinska fakulteta, Univerza v Ljubljani, Slovenija

Rak želodca je v Sloveniji pogosto maligno obolenje. Po podatkih "Registra raka za leto 1997" zavzema četrto mesto pri moških oziroma sedmo pri ženskah. Za njegov razvoj so odgovorne spremembe v številnih onkogenih in tumorje zaviralnih genih, poleg tega pri nekaterih oblikah tega raka zasledimo še mikrosatelitsko nestabilnost (MSI). Mikrosatelitski mutatorski fenotip je povezan s somatskimi premiki bralnega okvirja in točkovnimi mutacijami v nekodirajočih ponovljenih zaporedjih in kodirajočih regijah genov, povezanih z razmnoževanjem celic in programirano celično smrtjo.
Genetske spremembe v genu *hMLH1* in utišanje prepisovanja tega gena preko metilacije promotorja vodijo do pomankljivega delovanja celičnega popravljalnega sistema MMR.
V raziskavi smo pri bolnikih, pri katerih je bila odkrita MSI, s posrednimi in neposrednimi metodami iskali spremembe v genu *hMLH1*. Za posredno določanje sprememb smo uporabili metodo analize polimorfizmov enoverižnih DNA (SSCP), medtem ko smo vrsto in položaj mutacije določali z neposrednim določanjem nukleotidnega zaporedja. Pregledali smo vsa kodirajoča zaporedja (eksone), meje intron-ekson in krajši del nekodirajočih zaporedij, ki obdajajo posamezne eksone. Metilacijsko stanje promotorja tega gena smo določali z metodo restrikcije.
Odkrili smo sedem sprememb v nukleotidnem zaporedju gena *hMLH1*, od tega tri dedne mutacije in štiri dedne polimorfizme, pri dveh bolnikih pa smo odkrili metiliran promotor tega gena. Tri od omenjenih sprememb v literaturi še niso bile omenjene.

Mutation Analysis of *hMLH1* in Slovenian Patients with Gastric Cancer

P. Hudler
Faculty of Medicine, University of Ljubljana, Slovenia
Supervisor: S. Repše
University Clinical Centre Ljubljana, Slovenia
Co-supervisor: R. Komel
Faculty of Medicine, University of Ljubljana, Slovenia

Gastric cancer is one of the most common malignancies in Slovenia. According to data from "Cancer Registry for 1997" it is the fourth or seventh cause of cancer death form men and women, respectively. Alterations of multiple oncogenes and tumor suppressor genes, as well as microsatellite instability (MSI) are responsible for its carcinogenesis. Microsatellite mutator phenotype is the cause of many somatic frameshift and point mutations in non-coding repetitive sequences and coding regions associated with cell proliferation and apoptosis.
Genetic mutations in *hMLH1* and transcriptional silencing of its promoter by hypermethylation lead to inactivation of mismatch repair system.
In our study we performed screening for mutations in *hMLH1* at patients who expressed MSI genotype by using single-strand conformational polymorphism (SSCP) analysis and direct sequencing. To clarify the significance of *hMLH1* promoter hypermethylation we studied the methylation status of this promoter with restriction analysis.
SSCP and direct sequencing identified seven changes. Of these, three were germline mutations and other four were germline polymorphisms. In two cases we detected methylated *hMLH1* promoter. Three of the above alterations were described for the first time.

Etične dileme pri trženju zdravil brez recepta

D. Jaćimović
Ekonomska fakulteta, Univerza v Ljubljani, Slovenija
Mentor: M. Glas
Ekonomska fakulteta, Univerza v Ljubljani, Slovenija

Proizvajalci zdravil postajajo vse močnejši in pomembnejši, saj se vedno več ljudi zaveda pomena zdravega načina življenja in soglaša, da je bolje preprečiti kot zdraviti. Moj namen je bil prikaz stanja pri trženju zdravil brez recepta in nekatere etične dileme, ki se ob tem pojavljajo. Ponudniki se poskušajo uveljaviti s posebnimi oblikami promocij, z različnimi prodajnimi potmi, kar lahko predstavlja dileme za kupce in za zdravstvene oblasti, zato trženje samo po sebi nosi veliko težo, saj gre za odločilni člen povezovanja proizvajalca z bolnikom.
Zelo pomembno za uspešno tržno komuniciranje je poznavanje ciljne skupine potrošnikov. Tukaj se pokaže drugačnost farmacevtske industrije, kajti potrošnik (v našem primeru bolnik) nima možnosti izbire zdravila, ali pa je izbira precej omejena. Opravljena anketa je potrdila postavljene hipoteze o pomembnosti oglaševanja zdravil brez recepta za potrošnike. Za podjetje je še posebej pomembno, da je tržno komuniciranje, še posebej pa oglaševanje zdravil brez recepta zakonito, pošteno, etično, resnično in v skladu z želenim slovesom podjetja, katerega izdelek se oglašuje, da bo lahko izdelek dosegel tistega, ki mu je namenjen in mu pomagal do ozdravitve. Če bo zdravilo izpolnilo potrošnikovo - bolnikovo pričakovanje, se bo tudi v bodoče odločal za nakup teh zdravil (izdelkov) in oglaševanje bo doseglo svoj namen v okviru etičnih meril.

Ethical Dilemmas in Marketing of the OTC Drugs

D. Jaćimović
Faculty of Economics, University of Ljubljana, Slovenia
Supervisor: M. Glas
Faculty of Economics, University of Ljubljana, Slovenia

The drug producers are becoming more powerful and important because people are more aware of importance of being in good health and agree that it is better to prevent than to cure. My purpose was to show the situation in marketing of the OTC drugs and some ethical dilemmas, which may appear. The producers want to assert one with special forms of promotions, with various placements, which can present dilemmas for consumers and for health authorities so marketing itself caries a big responsibility, and is a crucial link to fasten together the producer and the patient.
Very important for successful promotion is to know whom the target consumer group is. The biggest difference between pharmaceutical companies and other producers is that the consumer (in our case patient) does not have the choice to choose the drug or the choice is very limited. The OTC drugs can be prescribed by doctors or can be bought in drug-stores where the patient is advised by a pharmacist and because of that a big share of promotion is orientated on the doctors and on the pharmacists on a highly professional level.
Performed inquiry has confirmed the hypothesises about importance of advertising OTC drugs for consumers. It is very important for the companies, that the promotion, especially advertising OTC drugs is legal, honest, ethical, truthful and in accordance with the wanted companies reputation. Promotion has to be clear for whom the product is advertised, so the product will reach the consumer and will help him get better. If the drug fulfils consumers - patients expectations, he will in the future also decide to buy that drug (product) and advertising will achieve its purpose within the framework of ethical standards.

Analiza prednosti in slabosti ter priložnosti in nevarnosti podjetja Krka

A. Jerina
Ekonomska fakulteta, Univerza v Ljubljani, Slovenija
Mentor: M. Bošnjak
Ekonomska fakulteta, Univerza v Ljubljani, Slovenija

Analiza prednosti in slabosti ter priložnosti in nevarnosti ali SWOT analiza je temelj, ki daje osnovo za strateško planiranje in postavljanje strateških planov v prihodnosti. Je ena od možnosti celovite ocene organizacije, kjer skušamo ugotoviti, kje ima ta določene prednosti in slabosti v primerjavi s konkurenčnimi organizacijami, ob tem pa iščemo tudi poslovne priložnosti in nevarnosti, ki se kažejo organizaciji v prihodnjem okolju na osnovi njenega dotedanjega gospodarskega potenciala, njenih sposobnosti in slabosti.
Analiza je pokazala, da so najpomembnejše prednosti v poslovanju Krke, d. d. visoko kakovostni proizvodi, visoka stopnja tržne prilagodljivosti, visoko usposobljen kader in kakovostno poslovodstvo, močna razvojna in investicijska dejavnost ter ustrezni finančni viri, ki zagotavljajo nadaljnji razvoj. Glavne slabosti, katerim mora podjetje posvetiti več pozornosti in jih z ustreznimi strategijami odpraviti, so toga organizacija podjetja, pomanjkanje managerjev za delo v tujini, premajhna usmeritev na zahodne trge, staranje kolektiva in poslovodstva, plačilna nedisciplina kupcev in pomanjkljiv sistem komuniciranja.
Okolje kaže podjetju priložnosti v večjem povpraševanju po generičnih zdravilih, ugodnih demografskih spremembah, novih možnostih raziskovanja zdravil, hkrati pa preti z nekaterimi nevarnostmi kot so harmonizacija farmacevtske zakonodaje z zakonodajo EU na tradicionalnih trgih, agresivnejši nastop tujih multinacionalk na tradicionalnih tržiščih, možnost sovražnega prevzema.
Krka naj gradi na konkurenčnih prednostih, ki ji omogočajo izpolnitev vizije in jo postavljajo v vrh generične farmacevtske industrije v srednji in vzhodni Evropi.

Analysis of Strengths, Weaknesses, Opportunities and Threats of Enterprise Krka

A. Jerina
Faculty of Economics, University of Ljubljana, Slovenia
Mentor: M. Bošnjak
Faculty of Economics, University of Ljubljana, Slovenia

The analysis of strengths, weaknesses, opportunities and threats or SWOT analysis gives the basis for the strategic planning as well as for the setting of such planes in the future. It is one of the ways of full assessment of the organization establishing its strengths and weaknesses in comparison with its competitors. On the basis of organization's economic potential, its capacity and drawbacks so far, we also try to find out its future business opportunities and threats.
The analysis has shown that the most important strengths of Krka are high quality products, high rate of market flexibility, well-qualified staff and quality management, strong developing and investment activity and adequate funds assuring further development of the company.
Rigid organization, shortage of managers for the work abroad, weak orientation on the Western markets, aging of staff and management, undisciplined payment system and unsatisfactory system of communication are the main weaknesses that the company should focus more on, trying to solve them with the appropriate strategies.
The environment offers opportunities in larger demand for generic drugs, favourable demographic changes, research opportunities of new drugs. However, Krka faces threats such as the harmonization of pharmaceutical legislation with EU`s legislation on its traditional markets, more aggressive approach of foreign multinationals on these markets as well as the possibility of a hostile takeover.
Krka should continue strengthening its competitive advantages, which enable the fulfillment of its vision and are placing the company on the top of generic pharmaceutical industry in the Central and Eastern Europe.

Vpeljava nove poti izobraževanja v podjetje s certifikatnim sistemom

T. Jović
Fakulteta za družbene vede, Univerza v Ljubljani, Slovenija
Mentor: I. Svetlik
Fakulteta za družbene vede, Univerza v Ljubljani, Slovenija
Somentorica: A. Pučko
Krka, d. d., Novo mesto, Slovenija

Zadnja leta se v naš prostor prebija prepričanje, da uspešnost vsakega podjetja temelji na kvalitetni izobrazbi in strokovni usposobljenosti ljudi. Hitre tehnološke spremembe zahtevajo široke izobraževalne programe, z znanji in spretnostmi, ki bi omogočale večjo mobilnost delovne sile in prenosljivost kvalifikacij v nova delovna okolja. Prvotno je bil certifikatni sistem opredeljen kot dopolnilo k šolskim spričevalom, v zadnjem obdobju se ga vse bolj obravnava kot integralni del formalnega izobraževalnega sistema. Gre za proces, kjer so izdani certifikati, ki formalno potrjujejo posameznikove dosežke v izobraževanju in izkazujejo kvalificiranost posameznika, ki si jo je pridobil izven formalnega šolskega sistema, kajti certifikatni sistem je vzporeden formalnemu šolskemu izobraževanju, pa vendar je od njega neodvisen. S certifikatnim sistemom se ukvarjajo druga telesa, izdajajo se posebna spričevala, prehodnosti v formalni šolski sistem ni in z njim si ni mogoče pomagati pri pridobivanju višjih stopenj izobrazbe. Namenjen je predvsem izpopolnjevanju in usposabljanju odraslih za opravljanje konkretnih del oziroma potrjevanju znanj, kvalifikacij in usposobljenosti, ki so jih posamezniki pridobili z opravljanjem določenih del ali na druge načine.
Podjetjem se z uvedbo certifikatnega sistema zmanjšajo stroški in čas (moduli) izobraževanja svojih delavcev, kar pelje do večje zaposljivosti. Posamezniki pa so s pridobitvijo certifikata bolj motivirani za delo, kar jim prinaša osebno zadovoljstvo in večjo uspešnost.

Introducing a New Way of Education into the Company with Certification System

T. Jović
Faculty of Social Sciences, University of Ljubljana, Slovenia
Supervisor: I. Svetlik
Faculty of Social Sciences, University of Ljubljana, Slovenia
Co-supervisor: A. Pučko
Krka, d. d., Novo mesto, Slovenia

In recent years, a belief that a success of each company is based on quality education and professional qualifications of people has spread around. Rapid technological changes require wider educational programmes, provisions of knowledge and skills, which enable larger mobility of the labour force and transferability of qualifications to new working environments. Originally the certification system was defined as the addition for school-leaving certificate, but in the last period of time it is considered as an integral part of formal school educational system. It is a process where certificates formally confirm the individual's accomplishments in education and are proving the qualifications of an individual that were gained out of formal school system. The certification system is parallel but still independent from general school system. Many others representative bodies are taking interest in certification system, special certificates has been granted, it isn't possible to transit to the formal school system and it isn't possible to gain higher lever of education with it. First of all it is meant for vocational education and training adults for doing real work and to certify the skills, qualifications and abilities which individuals had gained through their work or in other ways.
With the reform of certification system the companies can save up costs and time of company's workers (modules), which is leading to better employability. The individuals, who gained this certificate, are more motivated for work and can accomplish bigger satisfaction and work efficiency.

Analiza taktičnih značilnosti napada v košarki na osnovi trajektorij igralcev

M. Jug, J. Perš
Fakulteta za elektrotehniko, Univerza v Ljubljani, Slovenija
Mentor: S. Kovačič
Fakulteta za elektrotehniko, Univerza v Ljubljani, Slovenija
Somentor: B. Dežman
Fakulteta za šport, Univerza v Ljubljani, Slovenija

Opazovanje ljudi postaja čedalje pomembnejše podpodročje računalniškega vida, njegov najpomembnejši del pa je gotovo analiza gibanja ljudi.
Prvi del naloge je namenjen razvoju sistema za zajemanje človeškega gibanja, s poudarkom na sledenju igralcev v športnih igrah, med košarkarsko ali rokometno tekmo. Predstavljeni so problemi, povezani s pridobivanjem kvalitetnih podatkov med samo tekmo. Predlagane in preizkušene so bile ustrezne rešitve. Opisani so postopki računalniške obdelave posnetkov in način pridobivanja podatkov o pozicijah igralcev, ter postopek ovrednotenja sistema. Rezultat sledenja igralcev v košarki so trajektorije, ki opisujejo gibanje vseh igralcev skozi celotno tekmo.
V drugem delu je proučena možnost uporabe trajektorij igralcev za analizo taktičnih značilnosti košarkarskega napada. Struktura košarkarskega napada je predstavljena s stališča gibanja, določeni pa so tudi tisti deli trajektorij, ki predstavljajo ključne manifestacije taktičnih elementov. Razvit je bil model, s katerim je možno opisati stanja in dogajanje na igrišču.
Opisi treh tipov košarkarskega napada so bili uporabljeni za analizo trajektorij igralcev. Rezultati kažejo, da lahko na osnovi opaženih zvez ključnih dogodkov sklepamo ne samo na izvedbo tipa napada ali taktičnega elementa, ampak tudi na njeno kvaliteto.

Trajectory-Based Analysis of Basketball Offense's Tactical Properties

M. Jug, J. Perš
Faculty of Electrical Engineering, University of Ljubljana, Slovenia
Supervisor: S. Kovačič
Faculty of Electrical Engineering, University of Ljubljana, Slovenia
Co-supervisor: B. Dežman
Faculty of Sport, University of Ljubljana, Slovenia

'Looking at people' is one of the fastest growing areas of computer vision research, and human motion analysis is by far its most important discipline.
In the first part of the report, the development of the people tracking system for use in basketball and handball matches is presented. Problems, related to acquiring quality video data during the match were investigated. Appropriate solutions were proposed and tested. Methods of computerized video processing, which extract player positions from video, are presented and system evaluation is described.
In the second part of the report, a possible use of trajectory data in analysis of basketball offense's tactical properties is explored. The kinematical aspect of basketball offense's structure is presented and parts of trajectories representing key manifestations of tactical elements are identified. A model for representation of states and actions on basketball court is developed.
Representations of three types of basketball offense were used to evaluate player trajectories. Results show that relations of observed key events cannot only be used for action recognition but also to assess the quality of player performance.

Učinkovitost različnih načinov dezinfekcije biološko očiščene farmacevtske odpadne vode

B. Kalčič
Visoka šola za zdravstvo, Univerza v Ljubljani, Slovenija
Mentorica: E. Batagelj
Visoka šola za zdravstvo, Univerza v Ljubljani, Slovenija
Somentorici: I. Čarman
Krka, d. d., Novo mesto, Slovenija
T. Harlander
Zavod za zdravstveno varstvo Novo mesto, Slovenija

Namen raziskovalnega dela je bil ugotoviti bakteriološko kvaliteto biološko očiščene farmacevtske odpadne vode glede na slovensko zakonodajo ter določiti najustreznejši in ekološko neoporečen način dezinficiranja odpadne vode z natrijevim hipokloritom (NaOCl), vodikovim peroksidom (H_2O_2) in ultravijoličnim (UV) sevanjem. Učinek dezinficiranja je bil zasledovan na osnovi dokaza prisotnih indikatorskih mikroorganizmov. Za posamezno metodo dezinfekcije sta bila ugotovljena doza dezinficiensa in kontaktni čas, s katerima se je dosegel padec prisotnih indikatorskih bakterij v iztoku glede na zakonski predpis. V raziskavi so bile zasledovane tudi fizikalno - kemijske karakteristike (pH, temperatura, kemijska potreba po kisiku, neraztopljene snovi) biološko očiščene farmacevtske odpadne vode, ter vplivi navedenih oksidantov na inhibicijo vodnih bolh (*Daphnia magna Straus*). Bakteriološka slika biološko očiščene odpadne vode je pokazala, da je v iztoku povprečno število skupnih koliformnih bakterij 10^5/100 ml, koliformnih bakterij fekalnega izvora in fekalnih streptokokov pa 10^4/100 ml. Skupno število bakterij v iztoku je bilo 10^8, pogosto pa je bila prisotna tudi plesen. Primerjava učinkovitosti dezinficiensov in vpliva na inhibicijo vodnih bolh nakazuje, da je najmanj primerno dezinfekcijsko sredstvo vodikov peroksid. Kot najuspešnejša se je izkazala metoda hipokloriranja. V prihodnosti pa priporočam uporabo UV sevanja s predhodno filtracijo iztoka, oziroma izvajanje raziskav na področju membranske filtracije.

Efficiency of Different Disinfection Methods of Biologically Treated Pharmaceutical Wastewater

B. Kalčič
University College of Health Care, University of Ljubljana, Slovenia
Supervisor: E. Batagelj
University College of Health Care, University of Ljubljana, Slovenia
Co-supervisors: I. Čarman
Krka, d. d., Novo mesto, Slovenia
T. Harlander
Institute for Health Care Novo mesto, Slovenia

The main purpose of this research was to determine the bacterial quality of biologically treated wastewater as required by Slovene regulations. Sodium hypochlorite, hydrogen peroxide and ultraviolet radiation were used and compared. The disinfecting effect has been proven on the basis of the presence of indicating microorganisms. Disinfectant dose and necessary contact time have been determined for each disinfecting method, which achieved the decrease of the presence of indicator bacteria, according to regulation. Physical and chemical characteristics (pH, temperature, chemical oxygen demand, suspended solids) of biologically treated wastewater have also been determined as well as the influence of above-mentioned oxidants on the inhibition of water fleas (*Daphnia magna Straus*). The bacteriological picture of biologically treated wastewater showed that the average number of total coliform bacteria in the effluent was 10^5/100 ml, while the number of faecal coliform and faecal streptococci was 10^4/100 ml. The total number of bacteria in the effluent was 10^8, frequently with the presence of mould. Hydrogen peroxide was established as the least appropriate disinfectant. Hypochloring is the most successful. The most promissing methods are ultraviolet irradiation with the previous effluent filtration and the use of membrane filtration.

Azijske opcije

J. Kapetan
Ekonomska fakulteta, Univerza v Ljubljani, Slovenija
Mentor: D. Mramor
Ekonomska fakulteta, Univerza v Ljubljani, Slovenija

Leta 1973 so F. Black, M. Scholes in R. Merton na podlagi geometrijskega Brownovega gibanja oz. predpostavke o lognormalni porazdelitvi vrednosti osnovnega instrumenta, razvili prvi analitični model za vrednotenje opcij. Med najbolj popularnimi so azijske opcije, ki so se v današnji obliki prvič pojavile v devetdesetih letih prejšnjega stoletja. Njihova vrednost je močno odvisna od gibanja osnovnega instrumenta v nekem obdobju in ne zgolj od vrednosti le-tega ob dospetju (ang. strong path-dependent). Ključna je pot (gibanje cene) osnovnega instrumenta v opazovanem obdobju, na podlagi katere izračunamo povprečno vrednost cen osnovnega instrumenta v tem obdobju. Ker povprečja nihajo precej manj od posamičnih vrednosti, je finančni izid take opcije manj negotov, kar posledično vodi k nižji ceni opcije (in cenejšemu zavarovanju pred tveganji). Azijske opcije delimo na podlagi vrste povprečja, in sicer na aritmetične ter geometrijske. Vrednotenje prvih je izjemno kompleksno vprašanje, saj vsota lognormalno porazdeljenih spremenljivk ni lognormalno porazdeljena (pri geometrijskem povprečju teh težav ni). Zato analitična rešitev, ki je v primeru navadnih (Black-Scholes-Mertonov model) in geometrijskih opcij (ustrezna razširitev tega modela) razmeroma preprosta, za aritmetične azijske opcije (še) ne obstaja. Možnih, in v diplomskem delu natančno analiziranih, rešitev tega problema je več. Iz odnosa med aritmetičnim in geometrijskim povprečjem sledi, da je geometrijsko azijsko opcijo z določenimi prilagoditvami mogoče uporabiti za spodnjo mejo, sicer identične, aritmetične azijske opcije. Drugo možnost predstavljajo aproksimacije verjetnostne porazdelitve aritmetičnega povprečja s pomočjo lognormalne porazdelitve. Oboje da zadovoljive rezultate za nestanovitnosti osnovnega instrumenta do 30 % p.a. (sicer se verjetnostna porazdelitev aritmetičnega povprečja vse bolj odmika od lognormalne). Poleg tega je azijske opcije mogoče vrednotiti tudi s pomočjo binomskih dreves in Monte Carlo simulacij. Uporaba slednjih je priporočljiva, ko imamo opravka z nestanovitnostmi, ki so višje od omenjene.

Asian Options

J. Kapetan
Faculty of Economics, University of Ljubljana, Slovenia
Supervisor: D. Mramor
Faculty of Economics, University of Ljubljana, Slovenia

F. Black, M. Scholes and R. Merton developed the first closed-form formula for the value of an option in 1973. Among the most popular are Asian options. They are strongly path-dependent because their value prior to expiry depends on the path taken (average to date) and not just on where they have reached. Due to lower volatility of averages (in comparison to individual underlying values), the price of such option can be reduced significantly. There are two basic forms of Asian options based on the type of average used - arithmetic and geometric Asian options. The valuation of former is more complicated, since we cannot directly apply the methods normally used within standard Black-Scholes-Merton framework. That is due to the fact that the distribution of arithmetic average of a set of lognormal distributions does not have analytically tractable properties (on the other hand, the product of lognormal variables is itself a lognormally distributed variable and valuation of geometric Asian options is simply an extension of the original model). I analyzed different approaches to overcome this problem. The relationship between arithmetic and geometric averages enables us to, mutatis mutandis, use the corresponding geometric Asian option value as a lower boundary of arithmetic Asian option. The next set of approaches focuses on various approximations of the probability distribution of arithmetic average with lognormal distribution. They are satisfactory for underlying instruments' volatilities of up to 30 % p.a. For higher volatilities the difference between true and lognormal distributions becomes too large. Furthermore, numerical methods may be used, as well. I described the use of binomial trees and Monte Carlo simulations. The latter are recommended when dealing with volatilities higher than 30 % p.a.

Ekofiziologija črnih kvasovk redu *Dothideales* iz solin

M. A. Kocuvan
Biotehniška fakulteta, Univerza v Ljubljani, Slovenija
Mentorica: N. Gunde-Cimerman
Biotehniška fakulteta, Univerza v Ljubljani, Slovenija

Halofilne črne kvasovke *Hortaea werneckii*, *Phaeotheca triangularis*, *Trimmatostroma salinum* in halotolerantna črna kvasovka *Aureobasidium pullulans*, uvrščene v red *Dothideales*, predstavljajo prevladujoče vrste gliv, ki se pojavljajo v izjemno slanem okolju solin. Proučevali smo različne ekofiziološke lastnosti omenjenih črnih kvasovk, ki so ključne za rast ter za njihovo prisotnost v različnih mikronišah znotraj izjemno slanega habitata. Primarne ekološke mikroniše so lahko solinska voda, petola, les in biofilmi na različnih substratih. Proučevali smo adhezivne sposobnosti, prisotnost adhezivnega matriksa (eksopolimera) pri različnih pogojih, ugotavljali različne ekstracelularne encimske aktivnosti v slanih (5% in 17% NaCl) in neslanih pogojih ter potrdili prisotnost črnih kvasovk v vzorcih lesa z mikroskopom. Na podlagi dobljenih rezultatov smo sklepali o glavnih mikronišah posamezne vrste. Menimo, da so mikroniše halofilnih črnih kvasovk: solinska voda in predhodno razgrajena lesna vlakna za vrsto *Hortaea werneckii*, različne površine in biofilmi znotraj bazenov za vrsto *Phaeotheca triangularis*, in v slano vodo potopljen les za vrsto *Trimmatostroma salinum*. Halotolerantna vrsta *Aureobasidium pullulans* se pojavlja občasno in menimo, da zazvzema podobne mikroniše kot *Phaeotheca triangularis* (biofilme), vendar pri nižjih slanostih.

Ecophysiology of Black Yeasts (Order *Dothideales*) Isolated from Salterns

M. A. Kocuvan
Biotechnical Faculty, University of Ljubljana, Slovenia
Supervisor: N. Gunde-Cimerman
Biotechnical Faculty, University of Ljubljana, Slovenia

Halophilic black yeast-like fungi *Hortaea werneckii*, *Phaeotheca triangularis*, *Trimmatostroma salinum* and halotolerant *Aureobasidium pullulans*, all belonging to a single order *Dothideales*, are prevailing species of fungi found in extremely saline environment of the salterns. It has been assumed that these species possess characteristic enabling them to grow in different microniches of the hypersaline environment like hypersaline water, microbial mats, wood and biofilms on different substrates. In order to check the ecophysiological adaptations of the listed black yeasts to such environment, the following techniques were applied: [1] studies of adhesive ability and presence of adhesive matrix (exopolymer) in different conditions, [2] screening for different enzyme activities in saline (5 %, 17 %) and non-saline conditions, [3] determination of fungal presence in samples of wood with stereolens, light microscope and scanning electron microscope. Prevailing species isolated from wood submerged in hypersaline water were *Trimmatostroma salinum* and *Horteae werneckii*.
Finally, all of the above techniques lead us to speculate about the main microniches of the above listed species of black yeast-like fungi. We assume that the main microniches for halophilic species are: hypersaline water for *Hortaea werneckii*, biofilms on different surfaces for *Phaeotheca triangularis* and submerged wood for *Trimmatostroma salinum*. Halotolerant species *Aureobasidium pullulans* is not permanently present in extremely saline environment, but when appearing it occupies similar niches like *Phaeotheca triangularis* (biofilms).

Trženjski splet hotelov Otočec

K. Kočevar
Krka Zdravilišča; Krka, d. d., Slovenija
Mentor: M. Kučiš
Krka Zdravilišča, Krka, d. d., Slovenija
Somentorica: V. Žabkar
Ekonomska fakulteta, Univerza v Ljubljani, Slovenija

Da bi podjetje privabilo želeno količino turistov v njihov kraj, mora dodobra poznati njihove potrebe, želje in tako doseči njihovo čim višjo potrošnjo. Ker je v Sloveniji prisotnih že veliko zdravilišč, term, se morajo vedno znova usmerjati k individualizmu, visoki kakovosti storitev in konkurenčnim cenam.
Pokazala se je potreba po natančni opredelitvi trženjskega spleta posameznega zdravilišča. Nujno je bilo opredeliti storitve, ki jih bodo nudili in način kako doseči potencialne kupce, hkrati pa ustvariti pri tem uspešno poslovanje. V mojem diplomskem delu sem analizirala dosedanji trženjski splet Hotelov Otočec in se dotaknila novega, ki bo nastal po izgradnji novih vodnih površin. Do sedaj na Otočcu niso ponujali vodnih površin, gostom so to omogočili v 5 km oddaljenih Šmarjeških Toplicah, odslej pa bi jim to ponudili na samem kraju počitnikovanja. Poleg dveh obstoječih segmentov, bodo pridobili še nov segment, kajti v načrtu imajo tudi izgradnjo centra, ki bo med drugim ponujal tudi kardiovaskularne preglede. S tem bodo privabili ljudi, predvsem tiste, ki bi radi preživljali varne počitnice, pa tega ne želijo početi v zdravilišču.
Izvedla sem tudi anketo, ki sem jo statistično analizirala, postavila hipoteze, ki sem jih tudi dokazala in iz njih potegnila nekaj pomembnih ugotovitev. Glavni cilj ankete je bilo ugotoviti, kaj gostje najbolj pogrešajo v Hotelih Otočec. Kot predvidevano, največ gostov pogreša vodne površine. Zaradi novega kompleksa bi gostje pogosteje obiskovali Hotele Otočec in svoje bivanje tudi podaljšali.

Marketing Mix of Hotels Otočec

K. Kočevar
Krka Zdravilišča; Krka, d. d., Slovenia
Supervisor: M. Kučiš
Krka Zdravilišča, Krka, d. d., Slovenia
Co-supervisor: V. Žabkar
Faculty of Economics, University of Ljubljana, Slovenia

For a company to attract a desired quantity of tourists in its town, it is essential to know tourists needs and desires in order to achieve a higher consumption. A great number of spas and health centres in Slovenia forces them to orientate themselves to individuality, high level services and competitive prices. A need for an exact definition of marketing resolution of an individual health centre emerged. It was essential to define the offered services and how to reach potential buyers and at the same time create a successful management. The thesis presents the analysis of hitherto marketing mix of Otočec Hotels and touches the new one, which will appear after new water surfaces have been built. Otočec itself has so far not offered any water surfaces to its guests, but they were given the opportunity to use this particular service only 5 kilometers away in Šmarješke Toplice. But now the company wants to offer the service on the spot. Beside the two already existing segments, they will acquire a new one. Namely, their plan is to build a health centre offering preventative cardiovascular examinations. This will attract people, particularly those who want to have safe holidays but do not want to spend them in health centres.
I conducted a survey, which was statistically analysed, and I proposed hypotheses that I then confirmed and drew some important conclusions. The main goal of the survey was to establish what the guests miss in Otočec Hotels. The results showed they missed water surfaces. Due to the new complex the guests would visit Otočec Hotels more often and even prolonged their stay. This was proved by a hypothesis.

Približevanje Slovenije Evropski uniji ter vplivi na zdravstvo, trg zdravil in farmacevtsko industrijo

J. Korent
Ekonomska fakulteta, Univerza v Ljubljani, Slovenija
Mentor: B. Jazbec
Ekonomska fakulteta, Univerza v Ljubljani, Slovenija

Na slovenskem trgu zdravil se srečujeta Zavod za zdravstveno zavarovanje (ZZZS) in farmacevtska industrija. Vsak od njiju ima različne cilje, zato pride do nasprotujočih se interesov. ZZZS se sooča s pomanjkanjem sredstev za zdravstvo in vse večjimi izdatki za zdravila, farmacevtska industrija pa se razvija in raste prav na podlagi vse večjih izdatkov v zdravstvu in izdatkov za zdravila. Trend izdatkov za zdravila raste in je težko obvladljiv. Takšne razmere ne veljajo samo za naš trg, temveč se s podobnimi težavami srečujejo tudi drugod po Evropi. Države te težave rešujejo različno. Pri nas država določa cene zdravil in skuša omejiti samo porabo oziroma predpisovanje zdravil. Razmere v zdravstvu so predvsem posledica nejasnih stališč in ciljev vlade oziroma države.
Prav zaradi približevanja Slovenije EU delim mnenje s tistimi, ki zagovarjajo še boljšo zaščito domače farmacevtske industrije. Država bi s sprejetjem t.i. dobe podatkovne ekskluzivnosti lahko počakala do samega vstopa v EU. To bi predvsem vplivalo na boljša izhodišča Krke in Leka pri mogočem strateškem povezovanju ali prevzemu s strani večjih farmacevtskih podjetij.
Ugotovili smo, da se je Slovenija sicer začela zavedati pomena farmacevtske industrije, vendar po našem mnenju še premalo. Država pomaga farmacevtskim podjetjem s čim hitrejšim registriranjem zdravil in hitrim vpisom zdravil na liste. S tem hitro pridobijo dovoljenje za promet z določenim zdravilom. Trg zdravil pa bi naša država lahko še bolj zaprla, takšna praksa je tudi drugod po Evropi. S tem bi država še bolj zaščitila domači podjetji.

Slovenia Approach to EU and Impacts on Health Service, Medicine Market and Pharmaceutical Industry

J. Korent
Faculty of Economics, University of Ljubljana, Slovenia
Supervisor: B. Jazbec
Faculty of Economics, University of Ljubljana, Slovenia

There are two activists on Slovenian medicine market. On one side there is the Institution of Health Insurance (ZZZS) and on the other pharmaceutical industry. Each of them has different aims and that is the main reason why they cannot find mutual understanding. ZZZS is resourceless and the medicine expenditures are extremely high. Pharmaceutical industry is growing because of high health and medicine expenditures. That kind of problems is also present in the member states of EU. Countries are dealing with these problems by different methods. Our government is putting pressure on doctors not to prescribe so many medicines. Prices of medicines are also fixed. Unfortunately these methods are not successful.
I share an opinion with those who speak in favour of stronger and better protection of our pharmaceutical industry. Our government should have waited with accepting EU regulations on pharmaceutical industry, until we become a member state of EU. Krka and Lek would have a better possibility to become more stronger and successful international companies.
We established that Slovenia became aware of the importance of our pharmaceutical industry. State is helping the pharmaceutical industry with quick registrations of medicines and permissions to put medicines in the market, etc. State should also make medicine market more unapproachable for foreign pharmaceutical companies.

Validacija kromatografske analizne metode

J. Kos
Krka, d. d., Novo mesto, Slovenija
Mentorica: L. Zupančič-Kralj
Fakulteta za kemijo in kemijsko tehnologijo, Univerza v Ljubljani, Slovenija
Somentorica: J. Kunej
Krka, d. d., Novo mesto, Slovenija

Cilj validacije analizne metode je njeno ovrednotenje s statističnimi parametri in s tem potrditev, da je metoda primerna za določeno analizo. S tekočinsko kromatografijo smo validirali metodo določanja vsebnosti oksitetraciklina in sulfamonometoksin natrija v veterinarskem izdelku, ki se uporablja v veterinarski medicini za preprečevanje in zdravljenje želodčnih ter črevesnih obolenj in obolenj dihalnega sistema pri prašičih in govedu.
Glavni parametri pri validaciji metode so bili natančnost, linearnost, točnost, specifičnost/selektivnost in robustnost. Natančnost sem ovrednotila s ponovljivostjo instrumenta (HPLC sistema), ponovljivostjo metode in ponovljivostjo znotraj laboratorija. Pri linearnosti analizne metode smo ugotavljali, ali so merjeni signali v določenem koncentracijskem območju sorazmerni koncentraciji analizirane snovi v vzorcih. Točnost metode smo določevali z analizo vzorca, ki smo ga pripravili z odgovarjajočo količino placeba in z znano količino učinkovine. Pri specifičnosti/selektivnosti smo ugotavljali sposobnost analizne metode, da točno in specifično meri količino oz. koncentracijo preiskovane snovi v vzorcu v prisotnosti možnih dodatnih komponent, ki izhajajo iz učinkovine ali placeba. Robustnost analizne metode je sposobnost ohranjanja kvalitete rezultata pri majhnih spremembah parametrov metode in zagotavlja njeno zanesljivo uporabo. V okviru robustnosti smo preverjali stabilnost analiznih raztopin, vpliv filtriranja in vpliv rahlo spremenjenih analiznih pogojev.
Metodo za določevanje vsebnosti oksitetraciklina in sulfamonometoksin natrija smo uspešno validirali in dokazali, da je metoda primerna za rutinsko analizo.

Validation of Chromatographic Analytical Method

J. Kos
Krka, d. d., Novo mesto, Slovenia
Supervisor: L. Zupančič-Kralj
Faculty of Chemistry and Chemical Technology, University of Ljubljana, Slovenia
Co-supervisor: J. Kunej
Krka, d. d., Novo mesto, Slovenia

The aim of analytical method validation is an evaluation of the method by statistical parameters and a confirmation that the method is appropriate for a particular analysis. Liquid chromatography was used to validate the method for determination of the contents of oxytetracycline and sulfamonomethoxine sodium in a veterinary product, which is used to prevent and heal gastric and intestinal illness as well as illness of respiratory system at pigs and cattle.
The main parameters at validation were precision, linearity, accuracy, specificity/selectivity and robustness. I estimated precision with the help of system precision (HPLC system), method precision and intermediate precision. At linearity of analytical method we tried to establish whether the measured signals in a particular concentration area were proportional to the concentration of analyzed material in our samples. Method accuracy was estimated with the analysis of sample made of proper amount of placebo and of estimated amount of active substance. At specificity/selectivity we estimated the ability of analytical method to accurately and specifically measure the concentration of the substance under investigation in the sample where possible additional components are present, which are due to active substance or placebo. Robustness of analytical method is the ability to preserve the result quality with little changes of parameters and to assure its reliable use. We tested the stability of analytical solutions, the influence of filtration and the influence of slightly changed analytical conditions. With HPLC validation we proved that the method is appropriate for routine analysis and that very useful results can be obtained.

Uporaba večparameterskega hierarhičnega modela za pomoč pri odločanju o izboru vodilnega delavca

M. Košiček
Fakulteta za organizacijske vede Kranj, Univerza v Mariboru, Slovenija
Mentor: V. Rajkovič
Fakulteta za organizacijske vede Kranj, Univerza v Mariboru, Slovenija
Somentorica: N. Banovec
Krka, d. d., Novo mesto, Slovenija

V nalogi smo razvili model za pomoč pri odločanju, kjer smo upoštevali vse bistvene elemente razvoja kadrov. Pri gradnji hierarhičnega modela kriterijev za izbor vodilnega delavca, konkretno direktorja predstavništva v tujini, smo se oprli na tiste kriterije, ki so v podjetju Krka ključni za izbor kandidata.
Kot računalniško podporo smo uporabili program DEXi za večparametersko hierarhično modeliranje, ki pomaga pri odločanju med kandidati ter nudi možnost takojšnega vpogleda, zakaj je neki kandidat primeren in drugi manj primeren. Med primernimi kandidati lahko takoj ugotovimo, pri katerih kriterijih posamezniki izstopajo in kje ostajajo v senci ali pa so le povprečni. S tem pristopom smo prispevali k celovitemu, predvsem pa jasnejšemu pogledu na reševanje problema upravljanja s človeškimi viri.

Application of a Multi-attribute Hierarhic Model for Helping to Support a Decision for Selecting a Manager

M. Košiček
Faculty of Organisational Science Kranj, University of Maribor, Slovenia
Supervisor: V. Rajkovič
Faculty of Organisational Science Kranj, University of Maribor, Slovenia
Co-supervisor: N. Banovec
Krka, d. d., Novo mesto, Slovenia

In my diploma work I processed all the main elements of personnel development and of the expert systems and their implementation in everyday practice. The building of a hierarchic model of criteria for selecting a manager, in this case head of a representative office abroad, was based on Krka's essential personnel criteria.
We used DEXi as a computer support, which is used as a multi - attribute hierarchic model and is helping us in supporting a decision and give an instant view of the candidate's suitability. We can also determine his strong and weak areas.
Elaborating my diploma work helped us find out that expert systems, beside their basic intention to help in selecting, also provide a more complex, global and clearer view of the problem.

Iskanje gena za steroidno 11β-hidroksilazo pri nitasti glivi *Cochliobolus lunatus*

E. Kuščer
Biotehniška fakulteta, Univerza v Ljubljani, Slovenija
Mentor: R. Komel
Medicinska fakulteta, Univerza v Ljubljani, Slovenija

11β-hidroksilacija steroidov je ena ključnih stopenj pri proizvodnji kortikosteroidov. Encimi, ki so odgovorni za hidroksilacijo steroidov pri nitastih glivah, sodijo v encimsko naddružino citokromov P450. Kljub velikemu biotehnološkemu pomenu teh encimov pa doslej še niso uspeli izolirati genov, ki jih kodirajo. Tudi primarna struktura encimov še ni bila določena. Nitasta gliva Cochliobolus lunatus je pomemben rastlinski patogen s sposobnostjo 11β-hidroksilacije steroidov. V predhodnih raziskavah so na podlagi ohranjenih, za citokrome P450 značilnih zaporedij z metodo PCR pridobili 273 bp dolg genski odsek, kateremu so na podlagi primerjav v bazi podatkov BLAST potrdili naravo citokroma P450. Ta fragment smo uporabili za načrtovanje novih, za iskani citokrom P450 specifičnih začetnih oligonukleotidov. S temi smo z metodo hitrega pomnoževanja koncev komplementarne DNA iz induciranega micelija pomnožili celoten 3'-konec gena, kateremu pripada 273 bp dolg fragment, in del 5'-konca. 3'-koncu smo določili nukleotidno zaporedje, ki kaže visoko stopnjo homologije s številnimi citokromi P450. Pridobljeni fragment bomo v nadaljnjih raziskavah uporabili kot DNA-sondo za preiskovanje genske in cDNA knjižnice glive *C. lunatus*. Tako bomo odkrili celotni gen, kateremu pripada namnoženi fragment, z nekoliko nižjo intenziteto hibridizacije pa gotovo tudi druge citokrome P450, navzoče v tej glivi. Med pozitivnimi kloni bo nedvomno tudi tisti, ki vsebuje gen za steroidno 11β-hidroksilazo. Pridobljeni del 5'-konca je trenutno še v postopku določanja nukleotidnega zaporedja, vendar nam bo tudi ta razkril nove informacije in omogočil nove prijeme pri iskanju gena za steroidno 11β-hidroksilazo.

Searching for the Gene Encoding Steroid 11β-hidroxylase of the Filamentous Fungus *Cochliobolus lunatus*

E. Kuščer
Biotechnical Faculty, University of Ljubljana, Slovenia
Supervisor: R. Komel
Faculty of Medicine, University of Ljubljana, Slovenia

11β-hydroxylation of steroids is one of the key steps in the production of corticosteroids. The enzymes responsible for hydroxylation of steroids by filamentous fungi belong to the cytochrome P450 superfamily. Despite the substantial biotechnological importance of these enzymes, the sequences of the genes encoding any fungal steroid hydroxylase have not yet been identified, neither has their primary protein structure been described. The filamentous fungus Cochliobolus lunatus is a phytopathogen, capable of 11β-hydroxylation of steroids. Based on the conserved, cytochrome P450 specific regions, a 273 bp long gene fragment has been obtained using the PCR method. The fragment was sequenced, and the BLAST database search clearly showed its cytochrome P450 nature. Based on the sequence of the obtained fragment, new, cytochrome P450 specific primers were designed. Using the rapid amplification of cDNA ends method an entire 3'-end of the gene, and a part of the 5'-end were successfully amplified from the induced mycelium. The sequence of the 3'-end was determined, and using a BLAST database search, a high homology with numerous cytochromes P450 was shown. The obtained 3'-end will be used as a DNA probe for screening of genomic and cDNA libraries. Thus the entire gene to which the 3'-end belongs will probably be identified, as well as other cytochrome P450 genes present in the fungus. Among these, steroid 11β-hydroxylase will almost certainly be found as well. The acquired part of the 5'-end is currently being sequenced, and the information obtained will no doubt offer new approaches in searching for the gene encoding steroid 11β-hydroxylase.

Študij vpliva tipa in deleža razgrajevala na fizikalno - tehnološke lastnosti pelet izdelanih v hitrem mešalniku

T. Leskošek
Krka, d. d., Novo mesto, Slovenija
Mentor: R. Pišek
Somentor: F. Vrečer
Krka, d. d., Novo mesto, Slovenija

Izdelava ogrodnih pelet v hitrem mešalniku je za farmacevtsko industrijo zaradi razmeroma majhnih investicijskih stroškov zelo zanimiva.
V okviru raziskovalne naloge smo poskušali rešiti problem neustrezne razpadnosti pelet, ki vpliva na počasnejše sproščanje vgrajene zdravilne učinkovine. V hitrem mešalniku smo izdelali ogrodne pelete, v katerih smo varirali tip in koncemtracijo razgrajevala ter modelno učinkovino (dobro - slabo topno).
Namen raziskovalnega dela je bil ugotoviti in izbrati optimalno formulacijo z ustreznim tipom in deležem razgrajevala z namenom hitrega raztapljanja učinkovine ob sprejemljivih fizikalno - tehnoloških lastnostih izdelanih pelet.
Zaradi prisotnih razgrajeval, je izdelava pelet tehnološko zahtevnejša, saj peletna zmes nabreka že med samo izdelavo pelet. Težave se stopnjujejo z večanjem koncentracije razgrajevala. Povečevanje količine razgrajevala v trdnih farmacevtskih oblikah se kaže v slabih mehanskih lastnostih, trdnost upada in krušljivost narašča. Okroglost pelet se zmanjšuje s povečevanjem količine vključenega razgrajevala.
Najboljše rezultate smo dosegli z vgradnjo natrijevega karmezolata v 5% koncentraciji.

The Study of Influence of Disintegrant Amount and Type on Physico-technological Properties of the Pellets Produced in a High Shear Mixer

T. Leskošek
Krka, d. d., Novo mesto, Slovenia
Supervisor: R. Pišek
Co-supervisor: F. Vrečer
Krka, d. d., Novo mesto, Slovenia

Manufacturing of the matrix pellets in high shear mixer is for the pharmaceutical industry very interesting because of the small investment in comparison with other technologies.
Within the sphere of research work we try to solve the problem of inadequate disintegration of the pellets, which affect on slow release of the active substance. In high shear mixer we produced matrix pellets, in which we varied the type and the concentration of the disintegrator and model drug (good - poorly soluble).
The aim of the research work was to find out and choose the optimal formulation by using an appropriate type and amount of disintegrant to achieve the fastest dissolution of the drug, along with other acceptable physico-technological properties of the pellets produced.
The presence of disintegrants makes the production of pellets more technologically sophisticated, since the pellet mixture is swelling already during the manufacture itself. The difficulties become more intense with the increased amount of the disintegrant. The increased amount of disintegrant in solid pharmaceutical forms is showing poor mechanical properties, with decreased hardiness and increased friability. The sphericity of pellets decreases with an increased disintegrant amount.
We achieved the best results with incorporated croscarmellose sodium in 5 % concentration.

Možnosti zdravljenja aeromonasnih infekcij potočnih zlatovčic (*Salvelinus fontinalis*) s florfenikolom in oksitetraciklinom

M. Luštek
Veterinarska fakulteta, Univerza v Ljubljani, Slovenija
Mentor: V. Skubic
Veterinarska fakulteta, Univerza v Ljubljani, Slovenija

V moderni intenzivni vzreji postrvi povzročajo velike izgube kužne bolezni, kamor uvrščamo tudi furunkulozo ter druge aeromonasne infekcije. Ne glede na široko paleto antimikrobnih učinkovin, ki se v strokovni literaturi pojavljajo kot možna zdravila za uporabo v ribogojstvu, jih je za te namene zelo malo registriranih.
Ugotavljali smo učinkovitost novega antibiotika florfenikola (FF) in že uveljavljenega oksitetraciklina (OTC) pri zdravljenju aeromonasnih infekcij potočnih zlatovčic (*Salvelinus fontinalis*). V laboratoriju smo v nadzorovanih pogojih intraperitonealno okužili tri skupine potočnih zlatovčic z bakterijo *Aeromonas salmonicida subsp. salmonicida*. Takoj po poginu prvih rib smo pričeli z zdravljenjem. Prva skupina je dobivala OTC, druga FF, tretja pa le krmo brez antibiotika. Smrtnost zaradi furunkuloze je bila v skupini zdravljeni z OTC 96 %, v skupini zdravljeni s FF 68 %, v kontrolni skupini pa 95 %. Učinkovitost FF in OTC pri zdravljenju naravnega izbruha aeromonasnih infekcij smo ugotavljali tudi pri potočnih zlatovčicah v terenskem poskusu. Z zdravljenjem smo začeli hkrati v obeh skupinah. Ribe iz prve skupine smo zdravili z OTC, iz druge pa s FF. Ob koncu poskusa je bila skupna smrtnost v skupini zdravljeni z OTC 3,40 % in je bila signifikantno višja od skupine zdravljene s FF, kjer je poginilo 2,93 % vseh rib.
Ugotovili smo, da je florfenikol učinkovit antibiotik za zdravljenje aeromonasnih infekcij pri potočnih zlatovčicah, medtem ko so posamezni sevi bakterij iz rodu *Aeromonas* že odporni na oksitetraciklin.

The Possibilities of Treatment of Aeromonas Infections in Brook Trout (*Salvelinus fontinalis*) with Florfenicol and Oxytetracycline

M. Luštek
Veterinary Faculty, University of Ljubljana, Slovenia
Supervisor: V. Skubic
Veterinary Faculty, University of Ljubljana, Slovenia

Contagious infections, including furunculosis and the other aeromonas infections, cause considerable losses in the intensive fish farming. Although many antibacterial agents could be potentiality used in aquaculture, only few of them are approved.
The efficacy of a new antibiotic florfenicol (FF) and well-known oxytetracycline (OTC) against aeromonas infections in brook trout (*Salvelinus fontinalis*) was evaluated under the laboratory conditions and in the field trial, as well. Three groups of brook trout were intraperitoneally infected with *Aeromonas salmonicida subsp. salmonicida* under controlled conditions in a laboratory. The treatment started simultaneously in all test groups on the first day of mortality due to furunculosis. The first group received OTC and the second received FF. The fish from the third group received unmedicated feed only. Mortality due to furunculosis was 96 % in OTC group, 68 % in the FF group and 95 % in the control group. The efficacy of FF and OTC was also evaluated in the treatment of brook trout naturally infected with *Aeromonas spp.* in the field trial. Medication started simultaneously in both ponds, where the first group received OTC and the second group received FF. Mortality declined in both groups after a few days of medication. Cumulative mortality at the end of the trial was 3.40 % in the OTC group and 2.93 % in the FF group. Based on the results it can be concluded that FF is effective in treatment of aeromonas infections in brook trout. On the other hand resistance of some strains of *Aeromonas spp.* to OTC was perceived.

Določanje vpliva relativne vlažnosti na temperaturo steklastega prehoda felodipina z MTDSC

M. Medvešek
Fakulteta za farmacijo, Univerza v Ljubljani, Slovenija
Mentor: S. Srčič
Somentorica: N. Zajc
Fakulteta za farmacijo, Univerza v Ljubljani, Slovenija

V proizvodnji farmacevtskih oblik je zaradi izboljšanega raztapljanja in s tem biološke uporabnosti pogosto zaželeno steklasto stanje učinkovine. Sicer nestabilno steklasto stanje lahko opazujemo z določanjem temperature steklastega prehoda (T_g).
Preučevali smo vpliv relativne vlažnosti na steklast prehod felodipina. Hkrati smo ugotavljali ustreznost hidroksipropilmetilceluloze (HPMC) kot pomožne snovi za pripravo trdne disperzije, s katero bi stabilizirali steklasto obliko felodipina pri shranjevanju. Vzorce steklastega felodipina, HPMC in trdne disperzije felodipin/HPMC smo starali v eksikatorjih pri različnih relativnih vlažnostih. Po določenem času smo vzorce analizirali z diferenčno dinamično kalorimetrijo z modulacijo temperature (MTDSC), ki je nadgradnja klasične DSC. Steklast prehod smo ovrednotili s T_g in spremembo toplotne kapacitete (ΔC_p) z metodo polovice dviga krivulje na signalu "storage" C_p.
Ugotovili smo, da je znižanje Tg felodipina tem večje, čim večja je relativna vlažnost in čim dlje vzorec shranjujemo pri določeni vlagi. Rezultati so pokazali, da relativna vlažnost bistveno ne vpliva na T_g same HPMC, močno pa zniža temperaturo steklastega prehoda trdne disperzije. To je dokaz, da je izbira HPMC kot pomožne snovi za pripravo trdne disperzije neustrezna. Med shranjevanjem pri različnih relativnih vlažnostih ravno tako nismo opazili bistvene spremembe ΔC_p tako za felodipin in HPMC, kot za njuno trdno disperzijo.

Determination of Relative Humidity Influence on Felodipin Glass Transition Temperature Using MTDSC

M. Medvešek
Faculty of Pharmacy, University of Ljubljana, Slovenia
Supervisor: S. Srčič
Co-supervisor: N. Zajc
Faculty of Pharmacy, University of Ljubljana, Slovenia

In the production of pharmaceutical preparations the glassy state of active ingredient is often desired due to its increased dissolution rate and therefore improved bioavaliability. Generally unstable glassy state can be detected by determination the glass transition temperature (T_g).
We investigated the influence of relative humidity on felodipine glass transition. At the same time we examined the suitability of hydroxypropyl methylcellulose (HPMC) as excipient for solid dispersion preparation, which would stabilize the glassy felodipine during storage. Samples of glassy felodipine, HPMC and solid dispersion felodipine/HPMC were aged in desiccators at different relative humidity. After certain time, the samples were analysed using Modulated Temperature Differential Scanning Calorimetry (MTDSC), an extension of classical DSC. The glass transition was estimated with Tg and heat capacity change (ΔC_p) by means of half-step-height method on the "storage" C_p signal.
It was established that Tg of felodipine decreased with increasing relative humidity. Prolonged aging time at certain humidity indicated the same effect. Results showed no humidity influence on T_g of HPMC. However, the humidity influence on solid dispersion Tg was significant. This provides good evidence that HPMC is not suitable for preparation of felodipine solid dispersions. No considerable modification of ΔC_p for felodipine, HPMC and their solid dispersion was noticed during storage.

Sinteza sintonov antagonistov vitronektinskega receptorja

K. Nadrah
Fakulteta za farmacijo, Univerza v Ljubljani, Slovenija
Mentorica: M. Sollner Dolenc
Fakulteta za farmacijo, Univerza v Ljubljani, Slovenija

Vitronektinski receptor je v zadnjem času deležen velike pozornosti znanstvene javnosti, saj predstavlja povsem novo tarčo pri iskanju učinkovitejših načinov zdravljenja kardiovaskularnih obolenj, osteoporoze, revmatičnih obolenj, raka in infekcijskih stanj.
Pri pripravi antagonistov vitronektinskega receptorja se srečujemo z amidinsko skupino kot ključnim farmakofornim elementom.
Amidinsko skupino smo sintetizirali po dveh postopkih: s katalitičnim hidrogeniranjem amidoksimske skupine pri povišanem tlaku vodika in povišani temperaturi in s katalitičnim hidrogeniranjem 5-metil-1,2,4-oksadiazola, pri čemer se je prva v našem primeru izkazala za uporabnejšo.
Pripravili smo različne 3-substituirane 1,2,4-oksadiazolske derivate kot zaščitno skupino ali obliko predzdravila za amidinsko skupino.
Ugotavljali smo tudi stabilnost 1,2,4-oksadiazolske skupine v bazičnih pogojih.
Pripravljene spojine tako predstavljajo primerne sintone za sintezo novih antagonistov vitronektinskih receptorjev.

Synthesis of Sintons of Vitronectin Receptor Antagonists

K. Nadrah
Faculty of Pharmacy, University of Ljubljana, Slovenia
Supervisor: M. Sollner Dolenc
Faculty of Pharmacy, University of Ljubljana, Slovenia

Involvement of vitronectin receptor in various pathological states has been extensively reviewed in past few years. It's role in cardiovascular diseases, osteoporosis, rheumatoid disorders, cancer and infection renders it a new therapeutic target.
Amidine group is one of the key pharmacophores of ligand interaction with the vitronectin receptor.
We have prepared amidines by two methods: by catalytical hydrogenation of afore amidoxime and by catalytical hydrogenation of 5-methyl-1,2,4-oxadiazoline group. We found the former method far more effective.
We have also prepared various 3-substituted 1,2,4-oxadiazole derivatives as protecting groups or as prodrug forms for amidine group. We have studied the stability of 1,2,4-oxadiazole group in basic conditions.
Prepared molecules are suitable sintons for preparation of new vitronectin receptor antagonists.

Sinteza kompleksov platine(II) in platine(IV) z različnimi aminskimi ligandi

N. Perme
Fakulteta za kemijo in kemijsko tehnologijo, Univerza v Ljubljani, Slovenija
Mentorica: N. Bukovec
Somentorica: S. Grabner
Fakulteta za kemijo in kemijsko tehnologijo, Univerza v Ljubljani, Slovenija

Raziskave kompleksov platine kot potencialnih citostatikov so tako za medicino kot za farmacevtsko industrijo izrednega pomena. Cisplatin in karboplatina sta ena najbolj uporabljanih citostatikov na svetu. Vendar povzročata hude stranske učinke, delujeta le na določene oblike tumorjev, nekateri tumorji so nanju odporni, pri drugih pa povzroča razvoj odpornosti tumornih celic. Zato obstaja velik interes po razvoju novih kompleksov platine s širšim spektrom delovanja, izboljšano klinično učinkovitostjo in boljšo topnostjo.
Sintetizirali smo komplekse Pt(II) z dikarboksilnimi kislinami *cis*-[Pt(cbdca)(dach)]•H_2O, *cis*-[Pt(mal)(dach)], *cis*-[Pt(suk)(dach)]•H_2O, *cis*-[Pt(suk)(ipa)$_2$]•H_2O in *cis*-[Pt(cbdca)(ipa)2], kompleks Pt(II) s skvaratom *cis*-[Pt(skv)(ipa)$_2$]•H_2O in mešan kompleks Pt(II) z dvema različnima aminskima ligandoma *cis*-[PtI$_2$(hmpy)(ipa)]. Z oksidacijo *cis*-[Pt(mal)(dach)] smo dobili kompleks Pt(IV) trans-[Pt(OH)$_2$(mal)(dach)]. Sintetizirali smo tudi kompleks Pt(II) z aciklovirjem in sicer [Pt(acv-N7)$_2$(dach)]$^{2+}$.
Pri uspešnih sintezah smo optimizirali pogoje reakcije, da smo dobili čimbolj čist produkt s čimvečjim izkoristkom. Za nadzor poteka reakcij, identifikacijo produktov, ugotavljanje koordinacijske sfere ter čistosti dobljenih produktov smo uporabili rentgensko praškovno analizo, IR spektroskopijo, ^{1}H in ^{195}Pt NMR spektroskopijo ter termogravimetrično analizo.

Synthesis of Platinum(II) and Platinum(IV) Complexes with Different Amine Ligands

N. Perme
Faculty of Chemistry and Chemical Technology, University of Ljubljana, Slovenia
Supervisor: N. Bukovec
Co-supervisor: S. Grabner
Faculty of Chemistry and Chemical Technology, University of Ljubljana, Slovenia

Platinum complexes are interesting for medicine and farmaceutical industry because of their potential antitumor activity. Cisplatin and carboplatin are one of the most widely utilized antitumor drugs in the world. But they cause severe side effect, they affect only some forms of tumors, and some tumors have natural resistance while others develop resistance after the initial treatment. Because of these disadvantages there is a continuous need of new platinum drugs with broader spectrum of activity, better clinical efficiency and better solubility.
We synthesized Pt(II) complexes with dicarboxylic acids *cis*-[Pt(cbdca)(dach)]•H_2O, *cis*-[Pt(mal)(dach)], *cis*-[Pt(suk)(dach)]•H_2O, *cis*-[Pt(suk)(ipa)$_2$]•H_2O and *cis*-[Pt(cbdca)(ipa)$_2$], Pt(II) complex with s squarate *cis*-[Pt(skv)(ipa)$_2$]•H_2O and mixed Pt(II) complex with two different amine ligands *cis*-[PtI$_2$(hmpy)(ipa)]. Oxidation of *cis*-[Pt(mal)(dach) derived Pt(IV) complex trans-[Pt(OH)$_2$(mal)(dach). We also synthesized Pt(II) complex with acyclovir [Pt(acv-N7)$_2$(dach)]$^{2+}$.
After the successful synthesis we optimised reaction conditions in order to get pure products with optimal yield. We controlled course of reactions, identified the products and coordination sphere and determined purity of products by means of X-ray powder analysis, IR spectroscopy, ^{1}H in ^{195}Pt NMR spectroscopy and thermogravimetrical analysis.

Priprava cDNA sonde za TNFα

B. Premrov
Zavod RS za transfuzijo krvi, Ljubljana, Slovenija
Mentorica: V. Čurin-Šerbec
Somentorica: V. Galvani
Zavod RS za transfuzijo krvi, Ljubljana, Slovenija

Faktor tumorske nekroze alfa (TNFα) je pleiotropni vnetni citokin s širokim območjem delovanja. Kljub koristnemu sodelovanju TNFα pri obrambi gostitelja lahko njegova nenadzorovana sinteza vodi k nastanku patoloških in patofizioloških sprememb ter avtoimunskih procesov. Za kvalitativno in kvantitativno določanje mRNA za TNFα z metodo prenosa Northern se lahko uporablja cDNA sonda. Raziskavo smo izvedli na modelu celične linije človeških monocitov THP1, saj pri imunskih procesih *in vivo* največje koncentracije TNFα izločajo aktivirani monociti in makrofagi. Iz celic THP1 smo izolirali mRNA, RNA in proteine. Anti-TNFα monoklonska protitelesa E10/2, E2/2, F7/2 in C9/8 smo uporabili za izvedbo testa sendvič ELISA, s katerim smo določali celični TNFα in TNFα v supernatantu celic THP1. mRNA smo s pomočjo RT-PCR prepisali v ustrezno cDNA za TNFα, le-to pa pomnožili s PCR. Tako pripravljena cDNA sonda je bila velika 647 bp. S sekveniranjem smo potrdili njeno ustreznost. Sondo smo nato klonirali v plazmidni vektor pUC119. V ta namen smo izbrali par začetnih nukleotidov, s katerima smo TNFα sondi dodali dve novi restrikcijski mesti (*Eco*RI in *Hind*III) ter opravili kloniranje z lepljivimi konci. Konstrukt, ki je vseboval TNFα sondo, smo vnesli v kompetentne celice *E.coli* DH5α.
Pri našem delu nam je uspelo razviti sistem za pridobivanje velikih količin čiste cDNA sonde za TNFα z minimalnimi materialnimi stroški. Tako pripravljena sonda lahko služi kot orodje za spremljanje poteka tumorogeneze pri bolnikih z malignim melanomom.

Preparation of cDNA Probe for TNFα

B. Premrov
Blood Transfusion Centre of Slovenia, Ljubljana, Slovenia
Supervisor: V. Čurin-Šerbec
Co-supervisor: V. Galvani
Blood Transfusion Centre of Slovenia, Ljubljana, Slovenia

Tumor necrosis factor alpha (TNFα) is a pleiotropic inflammatory cytokine with wide range of actions. TNFα has a beneficial function in activation of host defense, its uncontrolled production can lead to pathological and pathophysiological consequences and autoimmune processes. A cDNA probe can be used for qualitative and quantitative analysis of mRNA for TNFα with Northern blotting.
The research was conducted on the human monocyte cell line THP1 as a model system since properly stimulated monocytes and macrophages present the major cellular source of TNFα *in vivo*. We isolated mRNA, RNA, and proteins from the cells. For detection of cellular TNFα and TNFα in supernatants of THP1 cells with sandwich ELISA we used anti-TNFα monoclonal antibodies E10/2, E2/2, F7/2 and C9/8. We performed RT-PCR with which we translated mRNA into cDNA for TNFα. With the use of PCR we multiplied the primary RT-PCR product. This cDNA probe was 647 bp long. By sequencing the probe we confirmed its adequateness. We cloned the cDNA into a plasmid vector pUC119. For this purpose we selected a couple of primers with which we introduced two new restriction sites (*Eco*RI and *Hind*III) into TNFα probe and performed cloning with sticky ends. We introduced the prepared construct into the competent *E.coli* DH5α cells.
In our research we have managed to develop a system for acquiring large quantities of pure probe with minimal material costs. This cDNA probe can be used as a tool for following the process of tumourogenesis in patients with malignant melanoma.

Študij vpliva velikosti delcev in specifične površine na izbrane tehnološko pomembne lastnosti modelne učinkovine KD-9102

M. Preskar
Krka, d. d., Novo mesto, Slovenija
Mentor: F. Vrečer
Krka, d. d., Novo mesto, Slovenija

Fizikalno-kemijske lastnosti surovin, ki vstopajo v dano formulacijo, so izrednega pomena, saj imajo velik vpliv na kakovost končnega izdelka.
V okviru raziskovalne naloge smo različnim serijam modelne učinkovine KD-9102 določili tehnološko pomembne fizikalne lastnosti, kot so tališče, entalpija taljenja, morfologija delcev, močljivost, higroskopnost, specifična površina, velikost delcev ter hitrost raztapljanja učinkovine.
Namen raziskovalnega dela pa je bil prikazati korelacijo med rezultati analiz, predvsem pa ugotoviti vpliv velikosti delcev in specifične površine na ostale fizikalne lastnosti, ki smo jih določili.
Iz rezultatov analiz sklepamo, da je v našem primeru velikost delcev odločilna lastnost proučevane učinkovine, saj le-ta vpliva na temperaturo tališča, močljivost in s tem tudi na konstanto hitrosti raztapljanja, medtem ko specifična površina, presenetljivo, nima vpliva na nobeno od navedenih lastnosti, ki smo jih določili, kar razlagamo s tendenco modelne učinkovine k aglomeraciji.
V okviru naloge smo proučevali tudi vpliv neodvisnih (čas gnetenja in količina tekočine za aglomeriranje) spremenljivk pri procesu granulacije KD-9102 v hitrem mešalniku na kakovost granulata.
Z eksperimentalnimi rezultati smo dokazali, da čas gnetenja v določenem intervalu vpliva le na konstanto hitrosti raztapljanja, medtem ko količina tekočine za aglomeracijo vpliva tudi na velikost granul.

Study of Influence of Particle Size and Particle Surface Area on the Technological Relevant Properties of Model Drug KD-9102

M. Preskar
Krka, d. d., Novo mesto, Slovenia
Supervisor: F. Vrečer
Krka, d. d., Novo mesto, Slovenia

It is known that physicochemical properties of raw material significantly influence the quality of the final pharmaceutical product.
Some technologically relevant physical properties (melting point, melting enthalpy, morphology, wettability, higroscopicity, particle size and dissolution rate) of different batches of model drug KD-9102 were determined.
The aim of the research was to show the correlation between the determined physical properties, but above all was to study the influence of particle size and particle surface area on the technologically relevant properties.
Analytical results indicated that particle size is the crucial property of KD-9102, which influences the melting point, wettability and constant of the dissolution rate of the drug.
Surprisingly, we have found out that particle surface area does not influence the determined properties, what was attributed to agglomeration of primary KD-9102 particles.
The influence of independent parameters in granulation process (kneading time and volume of agglomeration fluid) on the granules quality was also studied in present study.
Experimental results indicate that kneading time in certain interval influences only the constant of the dissolution rate, while a volume of the agglomeration fluid also influences the granule size.

Razvoj kapilarno elektroforezne metode za sledenje bilirubina in njegovih fotoizomerov v serumu

T. Pucelj
Fakulteta za farmacijo, Univerza v Ljubljani, Slovenija
Mentorica: J. Lukač-Bajalo
Somentor: S. Kreft
Fakulteta za farmacijo, Univerza v Ljubljani, Slovenija

Bilirubin je lipofilen naravni pigment, produkt presnove hemoglobina. Pri zdravem organizmu se v jetrih konjugira z glukuronsko kislino, da postane vodotopen, nato se z urinom in blatom izloči iz telesa. Če jetra niso dovolj razvita in nimajo še dovolj razvitega encimskega sistema za konjugacijo, se bilirubin ne izloča in se zadržuje v telesu, pride do rumenega obarvanja kože in beločnic, čemur pravimo zlatenica. Ta je še posebej nevarna pri novorojenčkih, ker lahko pusti trajne nevrološke posledice. Za zniževanje visoke koncentracije bilirubina se pri novorojenčkih večinoma uporablja fototerapija. Med fototerapijo se bilirubin izomerizira v topne netoksične fotoizomere. Čas izpostavljenosti modri svetlobi pa je odvisen od hitrosti zniževanja koncentracije bilirubina, ki jo dosežemo s fototerapijo. Ker ima kar nekaj neželenih stranskih učinkov, jo je priporočljivo pri novorojenčku uporabiti le kratek čas. Koncentracijo bilirubina je potrebno natančno spremljati, za kar pa imamo na voljo različne metode, a vsaka od njih ima svoje prednosti in slabosti.
Razvili smo kapilarno elektroforezno metodo za sledenje bilirubina in njegovih fotoizomerov v serumu, ki je enostavna, zahteva le majhne količine vzorca ter brez posebne predpriprave le-teh. Kombinacija parametrov: 50 mM tetraboratni pufer, 50 mM SDS, pH=9,3, način injiciranja s tlakom 40 mbar, čas injiciranja 25 s, redčenje vzorca z delovnim pufrom v razmerju 1:2 ter ustrezna napetost glede na dolžino kapilare, nam da optimalne pogoje za analizo. Detekcija poteka pri valovni dolžini 450 nm.

Development of a Capillary Electrophoretic Method for Monitoring Serum Bilirubin and its Photoisomers

T. Pucelj
Faculty of Pharmacy, University of Ljubljana, Slovenia
Supervisor: J. Lukač-Bajalo
Co-supervisor: S. Kreft
Faculty of Pharmacy, University of Ljubljana, Slovenia

Bilirubin is a liphofilic natural pigment, a product of haem metabolism. It conjugates normally in the liver with glucuronic acid, becomes polar and is easily excreted by urine and faeces. If the liver is not mature enough or its enzyme system for conjugation is not developed enough, bilirubin cannot be excreted, retains in organism and skin becomes yellow. This state is called icterus. It can leave permanent neurological consequences and can be especially very dangerous at newborns. Phototherapy is the most commonly used method to reduce high blood concentration of bilirubin. During phototherapy bilirubin converts to nontoxic polar photoisomers. Time of exposure to blue light depends of reducing concentration rate of bilirubin. Phototherapy has several side effects and must be used very rationally. Bilirubin concentration must be monitored and deteminated very exactly, because it is decided on the base of analytical results, how long the newborn needs to be exposed to the blue light. A few methods are available but they have their advantages and disadvantages.
We developed capillary electrophoretic method for monitoring serum bilirubin and its photoisomers, which is simple, needs only small amounts of sample and no special preparation of them. Combination of parameters (50 mM tetraboric buffer, 50 mM SDS, pH=9,3, injection with pressure 40 mbar, time of injection 25 s, diluting serum sample only with working buffer (1:2), appropriate voltage for each length of capillary, detection at 450 nm) gives us optimal analytical conditions for this method.

Analiza eksonov 1, 2 in 3 v genu za osteoprotegerin z metodo konformacijskih polimorfizmov enoverižnih DNA

T. Rajer
Fakulteta za farmacijo, Univerza v Ljubljani, Slovenija
Mentorica: J. Marc
Fakulteta za farmacijo, Univerza v Ljubljani, Slovenija

Osteoprotegerin (OPG) je nedavno odkriti protein, ki spada v družino receptorjev za dejavnike tumorske nekroze. OPG inhibira dozorevanje in aktivacijo osteoklastov, pospešuje njihovo apoptozo in s tem preprečuje izgubo kostne mase. Ker ima OPG pomembno vlogo v procesih kostne remodelacije, predstavlja njegov gen enega izmed kandidatnih genov, ki bi lahko bili odgovorni za razvoj osteoporoze. Polimorfizmi v tem genu bi lahko zato vplivali na vrednosti mineralne kostne gostote (BMD).
Z metodo konformacijskih polimorfizmov enoverižnih DNA smo analizirali odseke DNA z eksoni 1, 2 in 3 gena za OPG pri 61 pomenopavzalnih ženskah z osteoporozo. S sekvenčno analizo smo identificirali 5 polimorfizmov, od teh so trije še neobjavljeni. Ti polimorfizmi so: G1181C v eksonu 1, C1217T v intronu 1, G1284A v intronu 1, C4501T v intronu 2 in 4752_4753CT v intronu 3. Polimorfizem G1181C v eksonu 1 povzroči zamenjavo devete aminokisline lizin v asparagin.
S statistično analizo podatkov smo ocenili pogostost pojavljanja polimorfizmov (χ^2-test) in klinični pomen (ANOVA ali dvostranski t-test). Ugotovili smo da pri nobenem polimorfizmu ni razlik v frekvencah med osteoporoznimi bolnicami in zdravimi pomenopavzalnimi ženskami. Samo v primeru delecije 4752_4753CT smo dokazali, da imajo bolnice s spremenjenim genotipom (del/del) na obeh alelih glede na bolnice brez delecije (genotip CT/CT) višje vrednosti BMD ($p=0.016$). Pri ostalih polimorfizmih nismo dokazali povezanosti z vrednostmi BMD.
Na osnovi naših rezultatov lahko zaključimo, da je od petih genskih sprememb v začetku gena za OPG, funkcionalno pomembna le delecija 4752_4753CT. Homozigotne osebe s to spremembo imajo višjo BMD in manjše tveganje za razvoj osteoporoze.

Single Strand Conformation Polymorphisms Analysis of Exons 1, 2 and 3 of the Osteoprotegerin Gene

T. Rajer
Faculty of Pharmacy, University of Ljubljana, Slovenia
Supervisor: J. Marc
Faculty of Pharmacy, University of Ljubljana, Slovenia

Osteoprotegerin (OPG) is a recently discovered protein, a member of the tumor necrosis factor receptor family. OPG protects bone by inhibition of osteoclastogenesis, suppression of osteoclast activity, and induction of osteoclasts apoptosis. Because of its key role in the regulation of bone resorption the OPG gene is a possible candidate gene for osteoporosis. Sequence changes (polymorphisms) in this gene may influence bone mineral density (BMD).
We examined exons 1, 2, and 3 with the surrounding intron sequence of the OPG gene for polymorphisms in 61 pomenopausal osteoporotic women using SSCP analysis. Five different polymorphisms were identified using direct sequence analysis and three of them have not been published yet. These polymorphisms are: G1181C (exon 1), C1217T (intron 1), G1284A (intron 1), C4501T (intron 2) and 4752_4753CT (intron 3). G1181C causes the exchange of the third amino acid lysine to asparagine.
We statistically estimated the genotype frequencies of the informative polymorphisms (χ^2-test) and their association to BMD (ANOVA or two-side t-test). No differences in genotype frequencies between control group and pomenopausal women with osteoporosis were found. Only in case of deletion 4752_4753CT our data show significantly higher BMD in del/del genotype as compared to normal CT/CT genotype ($p=0.016$). No associations between other polymorphisms and BMD were found.
We can conclude that the deletion 4752_4753CT is the only functional DNA change in the first part of OPG gene. The women with del/del genotype have a higher BMD and reduced risk for osteoporosis.

Pregledovalnik mednarodne klasifikacije prakse zdravstvene nege na osebnem računalniku

U. Rajkovič
Fakulteta za organizacijske vede Kranj, Univerza v Mariboru, Slovenija
Mentor: J. M. Zupančič
Fakulteta za organizacijske vede Kranj, Univerza v Mariboru, Slovenija

Cilj naloge je bil razvoj programske opreme za pregledovanje Mednarodne klasifikacije prakse zdravstvene nege (International Classification of Nursing Practice - ICNP) v slovenskem in angleškem jeziku skupaj s programsko opremo za naložitev na osebni računalnik. V nalogi je predstavljena klasifikacija v okviru procesa zdravstvene nege in informacijskega sistema. Poseben poudarek je na podatkovnem modelu. Izdelana baza podatkov je poleg tega osnova tudi za rešitve pregledovalnika na svetovnem spletu in dlančniku. Vse tri rešitve smiselno dopolnjujejo knjižno verzijo klasifikacije. Programska rešitev je testirana v praksi in strokovno ocenjena. Kot taka je primerna za distribucijo na CD-romu končnemu uporabniku, praviloma medicinskim sestram in drugim zdravstvenim delavcem.

Browser of International Classification for Nursing Practice for Personal Computers

U. Rajkovič
Faculty of Organisational Sciences Kranj, University of Maribor, Slovenia
Supervisor: J. M. Zupančič
Faculty of Organisational Sciences Kranj, University of Maribor, Slovenia

The goal was to develop a software solution for browsing International Classification for Nursing Practice - ICNP in both Slovene and English language. The classification is presented in the frame of nursing process and information system. Special emphasis is on data model. The corresponding database is the basis not only for this browser but also for Internet and handheld computer (palm operating system) versions. These three solutions form together with a book a harmonized solution. Software has been tested in practice and evaluated by experts. As such it is ready to be distributed on CD-rom to the end-user - nurses and other health workers.

The Influence Of 3α,7α-dihydroxy-12-oxo-5β Cholanate on Insulin and Gliclazide Effect in Rats

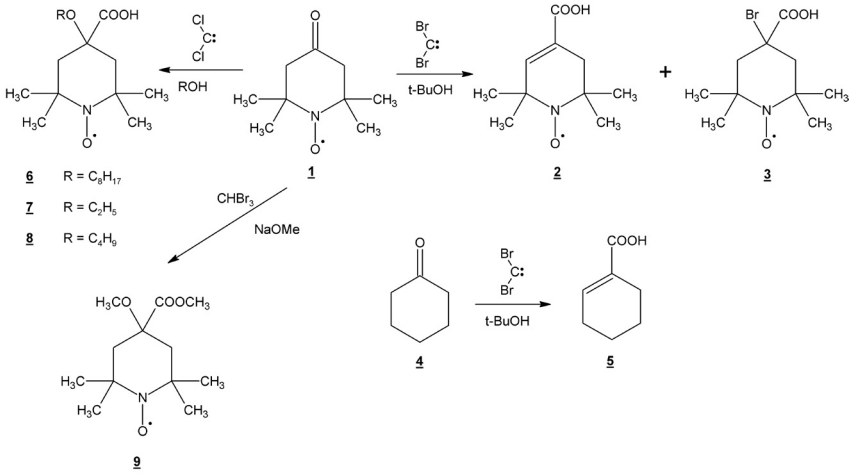
A. Rašković
Medical Faculty, Novi Sad, FR Yugoslavia
Supervisor: M. Mikov
Medical Faculty, Novi Sad, FR Yugoslavia

Besides well-known physiological properties of bile acids, the latest investigations have shown that their synthetic derivatives, sodium salt of 3α,7α-dihydroxy-12-oxo-5β cholanate (MCH), has significant influence on blood glucose levels. The aim of this study was to investigate the influence of MCH on hypoglycemic effect of insulin and gliclazide in normoglycemic and diabetic rats.
Diabetes was induced by streptozocin 75 mg/kg, b.w., i.p. Both groups of animals, diabetic and normoglycemic, were divided and treated with saline solution, intranasally and per os, 1 ml/kg b.w., MCH, 0.4 mg/kg, intranasally and orally, insulin, 10 i.u./kg b.w., intranasally, MCH and insulin combination, intranasally, gliclazide, 20 mg/kg b.w., orally, and MCH and gliclazide combination, orally. The animals were anaesthetised and blood glucose levels were measured during three hours after the treatment.
Groups treated with MCH had significant decrease of glucose blood levels only in diabetic rats. Insulin intranasally applied caused significant hypoglycemic effect in both groups, but this effect was significantly higher in diabetic rats. The treatment with gliclazide did not cause significant hypoglycemic effect, while gliclazide and MCH combination caused significant decrease of blood glucose levels in diabetic rats. MCH and insulin combinations caused significant hypoglycemic effect in comparison to control and insulin treated group. This effect was significantly longer in diabetic group.
On the basis of the results obtained, we can conclude that MCH is a potent hypoglycemic agent and that it significantly increases hypoglycemic effect of insulin and gliclazide in rats, which provides good basis for the further investigation of its antidiabetic effect.

Adicija dihalokarbena na karbonilno skupino v molekuli nitroksilnega radikala

T. Roblek, K. Naveršnik
Fakulteta za farmacijo, Univerza v Ljubljani, Slovenija
Mentor: S. Pečar
Fakulteta za farmacijo, Univerza v Ljubljani, Slovenija

Dihalokarbeni (halogen je Cl ali Br) so reaktivni intermediati, ki nastanejo iz kloroforma oz. bromoforma v bazičnem okolju. Medtem ko so adicije karbenov na alkene dobro opisane, pa so adicije na karbonilno skupino manj raziskane. Raziskovali smo možnosti pretvorb nitroksilnega radikala TEMPON-a (1) z dihalokarbeni. V reakcijah z diklorokarbenom smo sintetizirali 4-alkoksi-4-karboksi derivate (6), (7), (8) z izkoristki od 10 do 40%. Produkti reakcije z dibromokarbenom so bili ob uporabi natrijevega metilata 4-metoksi-4-metoksikarbonilni derivat (9), ob uporabi 50% NaOH kot baze pa 4-bromo-4-karboksilna kislina (3) in nenasičena kislina (2), slednja z izkoristkom 73%. Reakcijo smo izvedli tudi s cikloheksanonom (4), produkt je bil pričakovana nenasičena kislina (5) z izkoristkom 58%. Na osnovi naših ugotovitev lahko pripravimo nenasičeno kislino (2) v eni reakcijski stopnji z ugodnim izkoristkom.



Addition of Dihalocarbene to Carbonyl Group in a Molecule of a Nitroxyl Radical

T. Roblek, K. Naveršnik
Faculty of Pharmacy, University of Ljubljana, Slovenia
Supervisor: S. Pečar
Faculty of Pharmacy, University of Ljubljana, Slovenia

Dihalocarbenes are reactive intermediates, obtained from chloroform or bromoform in alkaline media. The addition of carbene into double bond has long been known, whereas the reaction with carbonyl groups is less documented. We studied the reaction of the carbonyl group in a nitroxide compound, TEMPONE (1), with dichloro- and dibromocarbene. We found that TEMPONE reacts with dichlorocarbene in the presence of certain alcohols, to produce compounds (6), (7), and (8) with yields ranging from 10% to 40%. Dibromocarbene produced compound (9) in the presence of sodium metoxide, while in the presence of 50% sodium hydroxide a mixture of 4-bromo-4-carboxy (3) and unsaturated acid (2) were obtained. Nitroxyl compound (2) can be obtained in 73% yield, while the yield of (5) was 58%. Now it is possible to prepare the unsaturated acid (2) in one step reaction from TEMPONE with acceptable yield.

Evropska farmacevtska industrija - analiza slovenske farmacevtske industrije

A. Sečnik
College of Europe, Brugge, Belgija
Mentor: R. Hine
College of Europe, Brugge, Belgija

Evropski farmacevtski trg daje možnosti in prostor obema vrstama industrij, torej inovativni in generični. Slovenska farmacevtska industrija je v zadnjem obdobju povečala svojo prisotnost na trgih EU, zato domnevamo, da je evropski regulatorni okvir zanjo zelo pomemben; še zlasti, ker je generična. V EU je generična industrija na osnovi sedaj veljavne patentne zakonodaje držav članic ovirana, saj izvajanje nekaterih aktivnosti (testiranje, klinično preskušanje) v času še veljavne patentne zaščite ni dovoljeno. V nalogi ugotavljamo, da pravna podlaga, ki je v skladu s TRIP-s sporazumom, generičnim proizvajalcem ne prepoveduje izvajanja določenih raziskovalnih aktivnosti še pred iztekom patentne zaščite. Zatorej bi morala biti obstoječa evropska patentna zakonodaja spremenjena, tako da bi dovolila izvajanje t. i. *klavzule Bolar*.
V empirični analizi smo ugotovili izraženo primerjalno prednost slovenske farmacevtske industrije glede na povprečje EU in v nekaterih, za Slovenijo, najpomembnejših farmacevtskih trgovinskih partnericah (Nemčija, Velika Britanija, Avstrija in Francija), in sicer pri tistih proizvodih (antibiotiki, rastlinski alkaloidi), kjer se je odvijala *znotraj panožna trgovina*. Trgovina z zdravili je potekala predvsem *medsektorsko*, tako da je Slovenija večinoma uvažala iz EU. Na osnovi kazalca *"vrednost na tono"* ugotavljamo, da se je na ravni celotne panoge odvijala *vertikalna, znotraj panožna trgovina* (Slovenija je menjala izdelke nižje kakovosti za izdelke višje kakovosti). Relativno visoki Grubel - Lloydovi indeksi izražajo podobno inovacijsko intenzivnost v Sloveniji in EU v nekaterih podsektorjih (antibiotiki, rastlinski alkaloidi), zato se odvija *znotraj panožna trgovina*.

European Pharmaceutical Industry - an Analysis of the Slovenian Pharmaceutical Industry

A. Sečnik
College of Europe, Brugge, Belgium
Supervisor: R. Hine
College of Europe, Brugge, Belgium

The European pharmaceutical market gives room to both types of industry, i.e. the proprietary and the generic. As the Slovenian pharmaceutical industry is generic the European regulatory framework deems to be relevant since it has recently intensified its performance in the EU market. As a matter of fact, the European generic pharmaceutical sector has still been somehow impeded since, under current patent legislation of Member States, pre-marketing activities (testing, clinical trials) are not allowed to be undertaken within the patent period. According to TRIPs, pre-patent research activities are allowed, therefore there is no need to hamper generic pharmaceutical sector with regard to the *Bolar provision* in the EU. Thus the EU patent legislation should be amended to allow generic companies to conduct development within patent expiry time.
As shown by the empirical analysis, revealed comparative advantage (RCA) was found in some products (Antibiotics, Vegetable Alkaloids) where the *intra-industry trade* (IIT) took place. Considering these products, the RCA was gained as for the EU average and with respective Member States (Germany, the UK, Austria and France) - the most important Slovenian trading partners in pharmaceuticals trade. Trading of medicaments between Slovenia and EU looks more or less *inter-industry*, meaning much more Slovenian imports from the EU. The *unit value ratio* indicates *vertical IIT* considering pharmaceuticals as a whole, assuming that Slovenia is trading with the EU medicinal products of lower quality in exchange for higher quality products from EU. Additionally, relatively high Grubel - Lloyd indices in the same sub-sectors (Antibiotics, Vegetable Alkaloids) suggest that rates of innovation are quite similar between Slovenia and the EU, hence *IIT* takes place.

Priprava plazmida CYP51-GFP za izražanje fuzijskega proteina med človeško lanosterolno 14α-demetilazo in zelenim fluorescinom v sesalskih celicah

M. Seliškar
Fakulteta za farmacijo, Univerza v Ljubljani, Slovenija
Mentor: B. Štrukelj
Fakulteta za farmacijo, Univerza v Ljubljani, Slovenija
Somentorica: D. Rozman
Medicinska fakulteta, Univerza v Ljubljani, Slovenija

Lanosterolna 14α-demetilaza (CYP51) je pri človeku vključena v poskvalenski del biosinteze holesterola. Nahaja se na citoplazemski strani endoplazemskega retikuluma (ER), kamor je zasidrana s svojim N-terminalnim koncem. Za CYP51 so dolgo mislili, da je stalno prisoten na ER in da zanj ni značilno kroženje z ER preko Golgijevega aparata nazaj na ER ali potovanje v ostale dele celice. Novejše raziskave kažejo, da je takšen transport v celici možen. Pripravili smo plazmid CYP51-GFP za izražanje fuzijskega proteina med človeškim CYP51 in zelenim fluoresceinom (GFP) v človeških celicah, ki nam bo v pomoč pri študiju znotrajcelične lokalizacije CYP51 in pri določevanje zaporedij CYP51, ki vplivajo na lokalizacijo. GFP, ki pri vzbujanju z UV svetlobo zeleno fluorescira, smo povezali s C-terminalnim koncem CYP51, ker je N-terminalni konec verjetno pomemben za usidranje v membrano ER in mora ostati prost. Pripravili smo ekspresijske plazmide za fuzijske proteine z različno spremenjenimi N in C-terminalnimi konci CYP51, da bomo lahko v kasnejšem raziskovalnem delu ugotovili vpliv teh sprememb na njegovo lokalizacijo. Enega od plazmidov smo vnesli v različne nesmrtne človeške celične linije in jih opazovali po 24 h, 48 h in 72 h pod konfokalnim fluorescenčnim mikroskopom. Izražanje fuzijskega proteina CYP51-GFP je bilo uspešno, saj smo opazili fluorescenco v območju citoplazme, ki označuje lokacijo CYP51, označenega z GFP. Za podrobno spremljanje znotrajcelične lokalizacije bomo morali pripraviti stabilne celične linije, ki bodo konstitutivno izražale fuzijski protein.

Preparation of CYP51-GFP Plasmid for the Expression of Fusion Protein Between Lanosterol 14α-demethylase and the Green Fluorescent Protein in Mammalian Cells

M. Seliškar
Faculty of Pharmacy, University of Ljubljana, Slovenia
Supervisor: B. Štrukelj
Faculty of Pharmacy, University of Ljubljana, Slovenia
Co-supervisor: D. Rozman
Faculty of Medicine, University of Ljubljana, Slovenia

Lanosterol 14α-demethylase (CYP51) is in humans involved in the postsqualene part of cholesterol biosynthesis. The enzyme is anchored with its N-terminus to the citoplasmic side of endoplasmic reticulum (ER). CYP51 was long believed to not cycle from ER through Golgi apparatus back to the ER nor to other parts of the cell. However, recent data indicate that recycling is possible between ER and Golgi apparatus. We prepared CYP51-GFP plasmid to express the GFP tagged CYP51 protein. This plasmid could be used to study intracellular localisation of the CYP51 and to determine which parts of this protein are important for its localisation. GFP which emits green fluorescence when it is excitated was fused to the C-terminus of CYP51 since the N-terminus is probably important for anchoring in the membrane of the ER and has to remain free. Expression plasmids for fusion proteins with different N and C-termini of the CYP51 have been constructed. The influence of these changes on CYP51 localisation will be studied in the future research work. Transfections of different immortal cell lines were performed with one of the plasmids. Cells were observed by confocal fluorescence microscope 24 h, 48 h and 72 h after transfection. The expression of fusion protein was successful since the green florescence was observed in the cytoplasm. This fluorescence indicates the localisation of the CYP51 tagged with the GFP. For more detailed studies of CYP51 intracellular localisation stable cell lines will have to be prepared which would constitutively express the CYP51-GFP fusion protein.

Gibanje "Slow food" v Sloveniji

B. Senekovič
Višja strokovna šola za gostinstvo Maribor, Slovenija
Mentor: M. Čurin
Hit Nova Gorica, Slovenija

Gibanje "Slow food" je bilo ustanovljeno v italijanskem mestu Bra v juliju 1986 kot protiutež gibanju "Fast foodu". Ustanovitelji so si za cilj zadali posredovati novo filozofijo okušanja kot kombinacijo harmonije hrane, pijače in užitka. S 65.000 člani na petih kontinentih in združenji v 45 državah je v trinajstih letih gibanje zraslo v mednarodno gibanje hrane in vina. "Slow food" tako postaja gibanje, ki ponuja drugačen odgovor glede napetega in stresnega življenje, ki spreminja naše življenje in okolje v katerem živimo. Svetovno gibanje je tudi v Sloveniji pognalo korenine, saj je vzpodbudilo zanimanje naših gurmejev in poznavalcev vin. Gibanje se je organiziralo po posameznih omizjih, ki jih vodijo konzuli. Tako ugotavljamo, da tudi pri nas gibanje "Slow food" temelji na ohranjanju lokalnih kultur, v prvi vrsti predvsem prehrambenih. Prav navade so smernice za uresničevanje idej gibanja in s tem tudi navad našega življenja. Opaža se, da je oblika ponudbe "Slow food" le nekakšna izhodišče za tiste, ki se te ponudbe poslužujejo na neamaterskem nivoju. Pri tem se opazi, da gre le za modni hit, ne pa za strokovno ponudbo.
Če želi gostinsko-turistično podjetje, ki bi se ukvarjajo s tovrstno ponudbo biti uspešno, si mora za tržno nišo izbrati tisti segment ljudi, ki uživajo v harmoniji hrane in pijače ter animirati ljudi, ki bi jih gibanje "Slow food" pritegnilo. Priložnosti se kažejo v vključevanju v potovalne aranžmane potovalnih agencij in turooperatorjev, ki bi gibanje "Slow food" vključili v del svoje ponudbe tako hotelov najvišje kategorije kot tudi zdravilišč.

"Slow Food" Movement in Slovenia

B. Senekovič
Higher Vocational School for Catering Maribor, Slovenia
Supervisor: M. Čurin
Hit Gorica, Slovenia

The "Slow Food" Movement was founded in the Italian town Bra in July 1986 as a response to the "Fast Food" movement. The founders of the movement wished to introduce a new philosophy of eating, as a combination of the harmony of food, drink and pleasure. With 65,000 members on five continents and organizations in 45 countries, the movement has in thirteen years become an international movement of food and wine. "Slow Food" is a movement offering an alternative attitude for changing our fast and stressful way of life, which has changed our way of being and threatens our environment and our landscape. This movement has also become popular in Slovenia among people with a passion for wine, food and related issues. The movement is organized in clubs called "tables", hosted by consuls. As in other countries "Slow Food" in Slovenia is dedicated to preserving the cultural heritage, especially local culinary traditions. "Slow Food" is all about restoring the conviviality of mealtimes to its rightful place in society as an international response to the effects fast food has on our society and life. Unfortunately, some restaurant owners, who offer it in an unprofessional way, consider "Slow Food" only a fashionable trend.
In order to run a successful business, a foodservice establishment offering "Slow Food" has to attract people who enjoy gathering around the table and take time to enjoy good food and wine. It should also animate people who would be interested in joining the "Slow Food" movement. There are also numerous possibilities for travel agencies and tour operators to include "Slow Food" in travel arrangements, as a part of the offer of hotels and health resorts of the highest category.

Razvoj HPLC-metode za določanje diklofenaka v sinovijalni tekočini

T. Sitar
Fakulteta za farmacijo, Univerza v Ljubljani, Slovenija
Mentor: V. Kmetec
Somentor: R. Roškar
Fakulteta za farmacijo, Univerza v Ljubljani, Slovenija

Diklofenak je kot nesteroidna protivnetna učinkovina zdravilo izbora za odpravljanje simptomov in zmanjševanje vnetja pri akutnem in kroničnem revmatoidnem artritisu. Biološka uporabnost diklofenaka po transdermalni aplikaciji gela je v sinovijalni tekočini kolenskega sklepa le 6%, minimalna efektivna koncentracija pa 100-500 ng/ml. Pod vplivom ultrazvočne masaže bi naj zaradi porušenja strukture kože prišlo do povečanja biološke uporabnosti. V ta namen smo razvili selektivno in dovolj občutljivo metodo tekočinske kromatografije visoke ločljivosti za določanje diklofenaka v sinovijalni tekočini. Kot optimalno smo izbrali reverzno fazno kolono C-18 z mobilno fazo s sestavo: 0,05 M fosfatni pufer pH 7,0 in metanol v razmerju 40/60 ter detektirali pri valovni dolžini 205 nm.
Najprej smo preizkusili stabilnost standarda in gela (Naklofen®) diklofenaka v prečiščeni vodi, kislini (0,001 M HCl) in bazi (0,001 M NaOH) pri sobni temperaturi in na ultrazvočni kadički. Za analizo diklofenaka v vzorcih sinovijalne tekočine smo razvili in optimizirali ekstrakcijski postopek, ki daje 90% izkoristke. Validacija analizne metode je potrdila, da je metoda selektivna, linearna v območju 30-500 ng/ml, ponovljiva in pravilna znotraj enega in treh dni. Meja zaznavnosti diklofenaka v sinovijalni tekočini je pri koncentraciji 25,4 ng/ml, meja določitve pri 77,1 ng/ml. Rezultati analiz realnih vzorcev potrjujejo, da je metoda dovolj občutljiva za študije določanja diklofenaka v sinovijalni tekočini kolenskega sklepa pacientov, ki jemljejo peroralni ali transdermalni pripravek.

Development of HPLC-method for Determination of Diclofenac in Synovial Fluid

T. Sitar
Faculty of Pharmacy, University in Ljubljana, Slovenia
Supervisor: V. Kmetec
Co-supervisor: R. Roškar
Faculty of Pharmacy, University in Ljubljana, Slovenia

Diclofenac is a nonsteroidal anti-inflammatory drug of first choice. It is indicated to prevent symptoms and decrease inflammation in therapy of acute and chronic rheumatoid arthritis. Bioavailability of diclofenac after transdermal application of gel is only 6% in synovial fluid of knee joint. The minimal effective concentration is estimated to be 100-500 ng/ml. We presume that ultrasound and massage might destroy skin structure and so enhance transdermal drug delivery. Selective and sufficient sensitive high performance liquid chromatography was developed for determination of diclofenac in synovial fluid. Diclofenac was analysed on reversed-phase C-18 column, using the mobile phase 0,05 M phosphate buffer pH 7,0 and methanol in the ratio 40/60. Detection was performed at 205 nm. At the beginning we investigated the stability of standard solution and gel (Naklofen®) of diclofenac in water, acid (0,001 M) HCl) and base (0,001 M NaOH) at room temperature and in ultrasonic bath. The extraction procedure for the determination of diclofenac in synovial fluid was developed and optimised to give 90% recovery. Validation of analytical method showed that developed procedure is selective, accurate, linear within 30-500 ng/ml with good repeatability and reproducibility. The limit of detection and quantification was 25,4 ng/ml and 77,1 ng/ml, respectively. Results of samples analyses confirmed the applicability of developed method for determination of diclofenac in synovial fluid of knee joint from patients who received oral and transdermal preparation.

Diclofenac for Surgical Treatment of Hydronephrosis in Children

V. V. Sizonov
Rostov State Medical University, Russia
Supervisor: M. I. Kogan
Rostov State Medical University, Russia

Introduction and objective: Anderson-Hynes operation is a standard surgical approach to treatment of ureteropelvic junction (UPJ) obstruction in children. However, 20-35% patients have disorders in urine outflow from a kidney, accompanied by pain and pyelonephritis, requiring prolonged kidney stenting. Causes of that phenomenon are complicated and are not fully clear. One of them is a severe blood circulation disturbance in ureter proximal end, arising after UPJ resection. The study objective is to study experimentally the ureter proximal end morphology following acute blood circulation disturbances in it, estimate diclofenac effect to the ureter restoration progress, and determine efficiency of postoperative diclofenac application in children in case of UPJ obstruction and hydronephrosis.
Materials and methods: During experiment UPJ ligation, ureterotomy, ureter suture were carried out in 20 rabbits at an age of 6 months, in 2 experimental groups: control and basic ones (postoperative diclofenac intramuscular introduction 2 mg/kg for 7 days). At 3rd, 5th, 7th day they were subjected to euthanasia and samples of UPJ were taken for histological examination.
243 children were subjected to surgery for hydronephrosis, diclofenac was introduced postoperatively to 60 patients followed by estimation of intrapelvic pressure.
Results: During the experiment (control group) development (3-7 days) of muscular fibre disorientation, intracellular and intermuscular edema, and early adventitia fibrosing were revealed. On the background of diclofenac introduction (basic group) at 7th day following the surgery edema is absent, leiomyocytes are preserved, and adventitia fibrosis is not developing.
Monitoring of intrapelvic pressure in children after UPJ resection showed reduction of intrapelvic pressure at 1st to 10th day on the background of diclofenac introduction as compared to the control group, where elevation of intrapelvic pressure was observed at 6-8th day. 22 patients were not subjected to pelvis drainage after UPJ plasty on the background of diclofenac treatment.
Conclusion: Diclofenac improves urodynamics of pelvis and new UPJ after elimination of obstruction. This allows to reduce number of postoperative complications, period of pelvis drainage, and in some cases to carry out UPJ drainage-free plasty.

Ali obstaja hitro rastoči rak materničnega vratu?

Š. Smrkolj
Univerzitetni klinični center, Ljubljana, Slovenija
Mentor: S. Rakar
Univerzitetni klinični center, Ljubljana, Slovenija

Incidenca raka materničnega vratu (RMV) je po letu 1994 v strmem porastu (od ustaljene incidence 15-16 / 100.000 žensk je porasla na 23 / 100.000 žensk leta 1997). Ta strmi porast ni samo posledica neodzivnosti žensk na preventivne preglede, ampak zelo verjetno tudi posledica večjega števila hitro rastočih RMV, ki naj bi nastali pri ženskah, ki hodijo na redne odvzeme brisov (v obdobju 1-3 let) in ki so še relativno mlade (30-40 let). Z namenom ugotoviti, ali obstoja hitro rastoči RMV tudi v Sloveniji, smo opravili analizo 624 bolnic z RMV, ki so bile vključene v Register raka RS v letih 1995-2000 tako, da smo ponovno ovrednotili citološke brise materničnega vratu in te primerjali s prvotnimi izvidi. Med 155 bolnicami, pri katerih so bili ponovno pregledani citološki brisi, smo ugotovili 9 (5.8 %) primerov bolnic, kjer je bil bris v zadnjih 3 letih negativen. 43 % bolnic ni hodilo na redne ginekološke preglede, pri redno pregledovanih bolnicah so bile prisotne napake v postopku zdravljenja in sicer očitne v 16 % primerov ter manjše v 31.5 % primerov. Pri bolnicah, ki so hodile na redne ginekološke preglede, so bili odvzeti citološki brisi materničnega vratu v 15.8 % lažno negativni, bodisi zaradi neustreznega odvzema (7.3 %) ali odčita (12.3 %). Glede na rezultate naše raziskave sklepamo, da je verjetno tudi v Sloveniji prisoten hitro rastoči rak materničnega vratu, resnične incidence hitro rastočega RMV ni mogoče ugotoviti, vsekakor pa je na podlagi naše analize delež bolnic s hitro rastočim RMV manjši, kot smo pričakovali in sicer okrog 5 %. Raziskava je opozorila na neustrezne postopke in slabšo kakovost dela tako ginekološke kot citološke stroke in na slabo osveščenost žensk, kar je pomembno za ustrezno programiranje detekcije RMV v sklopu Državnega programa za organizirano odkrivanje predrakavih sprememb materničnega vratu ZORA.

Does Interval Cervical Cancer Exist?

Š. Smrkolj
University Clinical Centre, Ljubljana, Slovenia
Supervisor: S. Rakar
University Clinical Centre, Ljubljana, Slovenia

After 1994 the incidence of cervical cancer has been dramatically increasing; the stabilized incidence of 15-16 / 100.000 women increased to 23 / 100.000 women in 1997. This steep increase might not be only the consequence of the women's negligence to come to preventive gynecological examinations, but very likely also the consequence of the actual higher number of interval cervical cancer cases. Interval cervical cancer is detected in women having the smear regularly taken (period 1-3 years) and being relatively young (30-40 years). In order to find whether interval cervical cancer exists also in Slovenia, we analysed 624 patients with cervical cancer, included in the Cancer Registry of the Republic of Slovenia in the period 1995-2000. We re-evaluated cytological smears taken from the uterine cervix and compared them to the initial ones. Of the 155 re-evaluated findings of cervical smears we found 9 (5.8 %) cases of interval cervical cancer. 43 % of patients did not come to regular gynecological examinations; in the patients coming to regular gynecological examinations, there were errors found concerning the prescribed treatment - in 16 % these errors were obvious, whereas in 31.5 % they were minor; besides, there were 15.8 % of false negative cytologic diagnoses, either for inadequate sampling (7.3 %) or inaccurate interpretation (12.3 %). The findings of this study confirm the existence of interval cervical cancer in Slovenia, but the incidence of this cancer cannot be estimated. However, the actual share of women with the rapid onset of cervical cancer is lower than expected (5 %). This analysis has brought up the issues of inappropriate procedures as well as inadequate quality of work in the fields of gynecology and cytology. On the other hand, it shows that women are still not sufficiently aware of the necessity of regular gynecological examinations, which is important for the realization of the National programme for detection of precervical lesions on the uterine cervix.

Biološka razgradnja odpadnega micelija glive rodu *Aspergillus*

M. Stražišar
Krka, d. d., Novo mesto, Slovenija
Mentor: A. Gasparič
Krka, d. d., Novo mesto, Slovenija

Odpadna micelarna pogača glive *Aspergillus terreus* var. *aureus* iz biosinteze lovastatina je po svoji kemijski zgradbi primeren substrat za biološko razgradnjo. Odpadek - inaktiviran micelij glive *Aspergillus* sestavljajo predvsem težje razgradljivi biopolimeri. Izhodiščne mikrobne vzorce za poskuse razgradnje micelarne pogače smo nabrali na različnih mestih v naravi, kjer smo pričakovali mikroorganizme, sposobne razgradnje naravnih polimerov. Namnožili smo bogatitvene kulture, ki smo jih skupaj z izhodiščnimi vzorci združili v pet novih vcepkov. Del teh smo vcepili v mešanice micelarne pogače z dodatki. Ugotavljali smo vpliv količine dodanih hranil in vcepka na razgradnjo micelarne pogače. Med razgradnjo vzorcev smo spremljali temperaturo mešanice, maso in pH-vrednost ter nadzorovali razgradnjo vzorcev. Rezultati kemijskih analiz razgrajene micelarne pogače in njihovih izlužkov kažejo na uspešnost razgradnje in kemijsko sorodnost razgrajenih vzorcev z gnojili in kompostom. Vsebnosti TOC, vsebnosti dušika v različnih oksidacijskih stanjih in KPK vrednosti so bile največje v izlužkih vzorcev, vzetih po biološki razgradnji. Z analizo TLC smo dokazali, da se je med biološko razgradnjo odpadka vsebnost ostanka lovastatina zmanjšala na približno 3,3% prvotne vsebnosti. Iz obogatenih kultur in razgrajenih vzorcev smo izolirali štiri združbe mikroorganizmov, ki so sposobne hitre in popolne razgradnje peletne oblike glive *A. terrreus* var. *aureus*, inhibicije kalitve spor in uspešne razgradnje odpadne micelarne pogače. Rezultati raziskave kažejo, da je odpadna micelarna pogača iz proizvodnje lovastatina z izoliranimi združbami mikrobov biološko razgradljiva, s tem pa se odpira možnost uporabe razgrajene odpadne micelarne pogače kot gnojilo ali kompost.

Biological Degradation of Waste Mycelium Fungi Genus Aspergillus

M. Stražišar
Krka, d. d., Novo mesto, Slovenia
Supervisor: A. Gasparič
Krka, d. d., Novo mesto, Slovenia

Waste mycelial cake of *Aspergillus terreus* var. *aureus* from lovastatin biosyntesis is due to its chemical structure appropriate substrate for biological degradation. The waste - inactivated *Aspergillus* mycelium is mostly composed of relatively resistant biopolymers. Original microbial cultures isolated and prepared for biodegradation experiments have been collected at different places in environment, where microbes, capable of natural biopolymers degradation, were expected. Enrichment media with mycelial cake have been developed and microbial cultures grown merged together with original samples into five combined inocula. Mixtures of mycelial cake and additives were inoculated with these inocula. We tested the influence of the quantity of added nutrients and inocula on degradation process of the waste mycelial cake. Temperature, mass, pH-value and degree of degradation have been monitored. Chemical analyses proved a successful degradation and chemical similarity of decomposed samples to fertilizers and composts. TOC amounts, concentration of nitrogen compounds and COD values in the extracts of biologically treated samples were the highest at the end of biodegradation process. TLC analysis proved that the concentration of the residual lovastatin was during the biodegradation process of mycelial waste reduced to the concentrations lower than 3,3% of the initial amount. 4 different microbial communities were isolated from enrichment cultures and biologically decomposed mixtures of mycelial cake, that were capable of quick and total degradation of *A. terreus* var. *aureus* pelets, inhibition of germination of fungal spores and successful decomposition of mycelial cake. Our research results pointed out that waste mycelial cake from production of lovastatin could be successfully decomposed by isolated microbial communities and potentially used as fertilizer.

Dokazovanje prisotnosti bakterij *Staphylococcus aureus* in *Pseudomonas aeruginosa* v zdravilih z verižno reakcijo s polimerazo

A. Škof
Zavod za farmacijo in za preizkušanje zdravil, Ljubljana, Slovenija
Mentor: A. Krbavčič
Fakulteta za farmacijo, Univerza v Ljubljani, Slovenija
Somentor: M. Poljak
Medicinska fakulteta, Univerza v Ljubljani, Slovenija

V zdravilih za dermalno uporabo in uporabo na sluznicah je predpisana odsotnost bakterij *Staphylococcus aureus* in *Pseudomonas aeruginosa*. S standardno mikrobiološko metodo potrebujemo za osamitev in identifikacijo obeh bakterij od 5 do 7 dni, zato se pojavlja potreba po novih tehnikah, ki bi bile hitrejše.
Namen naloge je bil razviti metodo verižne reakcije s polimerazo (PCR), s katero bi lahko hitreje dokazali prisotnost majhnega števila navedenih bakterij v tovrstnih zdravilih.
Vzorce smo predhodno bogatili 24 ur, s čimer smo povečali občutljivost metode. Bakterijsko DNA smo osamili s komercialno dostopnim reagenčnim kompletom High Pure PCR Template Preparation Kit (Roche). Tarčno sekvenco bakterijskega genoma smo dokazovali z metodo PCR v realnem času s sistemom LightCycler™ (Roche), ki omogoča sočasno pomnoževanje in dokazovanje pridelka PCR s pomočjo hibridizacijskih lovk. Detekcija s hibridizacijskimi lovkami je zelo specifična in ne zahteva dodatne potrditve specifičnosti pridelka PCR.
Uporabnost metode PCR za dokazovanje prisotnosti oz. odsotnosti bakterij *Staphylococcus aureus* in *Pseudomonas aeruginosa* v zdravilih za dermalno uporabo in uporabo na sluznicah smo uspešno dokazali pri 34 vzorcih (mazila, kreme, geli, emulzije in raztopine).
Z uporabljeno metodo lahko dokažemo prisotnost majhnega števila bakterij (1-10 CFU/g) v 28 urah, kar pomeni v primerjavi s standardno mikrobiološko metodo velik prihranek časa.

Detection of *Staphylococcus aureus* and *Pseudomonas aeruginosa* in Medicaments by Polymerase Chain Reaction

A. Škof
Institute of Pharmacy and Drug Research, Ljubljana, Slovenia
Supervisor: A. Krbavčič
Faculty of Pharmacy, University of Ljubljana, Slovenia
Co-supervisor: M. Poljak
Faculty of Medicine, University of Ljubljana, Slovenia

In medicaments for topical use the absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa* is required. For isolation and identification of both bacteria by standard microbiological method 5 to 7 days are required. Therefore there is a need to develop and apply new technologies, which will be more rapid.
The purpose of this work was to develop a PCR assay for faster detection of low levels of both bacteria in medicaments for topical use.
Sample pre-enrichment was used to improve the sensitivity of method. After 24-hour enrichment bacterial DNA was isolated with the High Pure PCR Template Preparation Kit (Roche). Selected target sequence of bacterial genome was detected by a real-time PCR. The PCR assay was performed in a LightCycler™, which enables simultaneous amplification of target sequence and detection of PCR product with hybridization probes. Detection with hybridization probes is highly sequence specific and does not require any additional procedure to confirm the specifity of PCR product.
Applicability of PCR assay for determination of presence or absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa* in medicaments for topical use was successfully proved in 34 samples (ointments, creams, gels, emulsions and solutions). Low level of bacteria could be detected by PCR in only 28 hours. Compared to the standard microbiological method this means a great saving in time.

Pitovne in klavne lastnosti kopunov

B. Štamcar
Biotehniška fakulteta, Univerza v Ljubljani, Slovenija
Mentorica: A. Holcman
Biotehniška fakulteta, Univerza v Ljubljani, Slovenija

Kopunje meso je nekdaj veljalo za specialiteto, ki se je uporabljala le za posebne priložnosti. Zaradi drage reje so kopune izpodrinili s tržišča ceneni pitovni piščanci. Nekdanji sloves in zanimanje za oživljanje reje kopunov je vzpodbudilo proučitev pitovnih in klavnih lastnosti kopunov. V poskus smo vključili 39 kopunov in delnih kopunov prelux-G ter 38 kopunov in delnih kopunov slovenske avtohtone pasme kokoši štajerke. Kopune smo zrejali pri treh rejcih v hlevih z izpusti od 11. do 33. tedna starosti. Telesne mase kopunov in delnih kopunov se pri tehtanjih niso značilno razlikovale. Kopuni in delni kopuni štajerske pasme so bili lažji od prelux-G kopunov in delnih kopunov. Klavne lastnosti so bile merjene na vzorcu devetih kopunov in šestih delnih kopunov prelux-G ter šestih kopunov in treh delnih kopunov štajerske pasme. Telesna masa pred klanjem, trebušna zamaščenost in masa klavnih trupov kopunov je bila značilno večja kot pri delnih kopunih. Prelux-G kopuni so v primerjavi s kopuni štajerske pasme dosegli značilno večje vrednosti pri telesni masi pred zakolom, masi klavnih trupov in klavnosti. Med pasmama ni bilo značilnih razlik v zamaščenosti trupov. Barva kože med kopuni in delnimi kopuni se je značilno razlikovala v svetlosti, rdečini in rumenosti (CIE L, a, b vrednosti).

Fattening Performance and Carcass Traits of Capons

B. Štamcar
Biotechnical Faculty, University of Ljubljana, Slovenia
Supervisor: A. Holcman
Biotechnical Faculty, University of Ljubljana, Slovenia

Capons meat used to be a speciality, prepared only for special occasions. Due to the expensive fattening process it has been replaced by the cheap market broiler. Because of the former capon reputation, very few literature information and possibilities to bring the fattening to life again, we carried out a research on capon's fettening performance and carcass traits. 39 capons and slips of Slovenian provenance Prelux-G together with 38 capons and slips of Slovenian autochthonous Styrian breed (partridge variation) were included into the research. The capons were bred in poultry houses on three farms and were left outdoors between 11th and 33rd week of age. The weight of capons and slips did not differ substantially at any age. The capons and slips of Styrian breed were lighter than the Prelux-G. Carcass traits were measured on the sample of nine capons and six slips of Prelux-G and six capons and three slips of Styrian breed. At slaughter body weight, fat and carcass weight of capons was significantly higher than in slips. In comparison to Styrian breed, Prelux-G had a considerably higher body weight, carcass weight and dressing percentage. As far as fat is concerned there were no significant differences between the breeds. Skin colour between the capons and slips was differed in brightness, redness and yellowness (CIE L, a, b values).

Ocena obratovalnih pogojev med fermentacijo salinomicina

P. Štukelj
Krka, d. d., Novo mesto, Slovenija
Mentor: V. Koloini
Fakulteta za kemijo in kemijsko tehnologijo, Univerza v Ljubljani, Slovenija
Somentor: I. Radež
Krka, d. d., Novo mesto, Slovenija

Želja inženirja v industriji je, da na podlagi meritev, opravljenih na manjših pilotnih napravah, napove potek fermentacije v industrijskih fermentorjih. Žal pa to ni mogoče pri vseh parametrih, saj veliko dejavnikov vpliva nanje. Meritve viskoznosti podajajo različne rezultate v pilotnih in industrijskih fermentorjih.
Dodajanje vode med vodenjem fermentacije povzroča zmanjšanje viskoznosti zaradi razredčitve v pilotnem in industrijskem fermentorju. Tako dodatek 10 % vode pomeni zmanjšanje efektivne viskoznosti za 45%. Vendar viskoznost močno naraste v naslednjih treh urah kot posledica dodajanja vode. Na rezultat meritve viskoznosti vpliva v obeh primerih tudi čas, ki preteče od vzorčenja do meritve. Rezultati potrjujejo, da moramo meritev opraviti v prvih desetih minutah, v nasprotnem se pojavi več kot 10% napaka.
Pomemben parameter je tudi vnos moči med fermentacijo in kontrola različnih korelacijskih enačb, ki napovedujejo vnos moči. Meritve, opravljene na 65 in 80 m^3 fermentorjih pri mešanju s tremi Rushtonovimi mešali, so potrdile ustreznost uporabe konstante števila moči 5,5 za Rushtonovo turbino in pokazale sprejemljivo ujemanje z Millerjevo korelacijsko enačbo.
Teoretično sem ocenjeval tudi mešalne pogoje v 65 in 80 m^3 fermentorjih in ugotovil, da v začetku obratujemo v območju "flooding", ko mešalo zraka ne dispergira. Nadalje fermentacija poteka v območju "loading", ko mešalo zadovoljivo dispergira tok uvajanega zraka.

The Assessment of the Operational Conditions During the Fermentation of Salinomicine

P. Štukelj
Krka, d. d., Novo mesto, Slovenia
Supervisor: V. Koloini
Faculty of Chemistry and Chemical Technology, University of Ljubljana, Slovenia
Co-supervisor: I. Radež
Krka, d. d., Novo mesto, Slovenia

The wish of an industry engineer is to foresee the course of fermentation in the industrial fermentors on the basis of the measurements taken on smaller pilot devices. However, this is not possible with all parameters because many factors influence these parameters. The viscosity measurements give different results in pilot as well as industrial fermentors.
The addition of water during fermentation causes the decline in viscosity due to dilution in the pilot and industrial fermentor. Thus adding 10 % of water causes the decline in effective viscosity for 45 %. However, there is a sharp rise in viscosity in the next 3 hours due to water addition. The result of the viscosity measurements is in both cases influenced by the time-span from sampling to measuring. The results have confirmed that the measurements have to be taken in the first ten minutes, otherwise we get more than a 10 % error.
An important parameter is also the input of power during fermentation and the control of different correlative equations that predict the input of power. The measurements taken in 65 m^3 and 80 m^3 fermentors, using three Rushton turbine, have confirmed the use of the power constant 5.5 for the Rushton turbine and they have shown a variable analogy with the Miller correlative equation.
Theoretically I have also assessed the mixing conditions in the 65 m^3 and 80 m^3 fermentors; my conclusion is that in the beginning we operate in the "flooding" area when the mixing device doesn't disperse the air. Afterwards the fermentation continues in the "loading" area when the mixing device sufficiently disperses the current of the input air.

Internacionalizacija farmacevtskih družb na trgih vzhodne Evrope

D. Šuštaršič
Ekonomsko-poslovna fakulteta, Univerza v Mariboru, Slovenija
Mentorica: Z. Bajc
Krka, d. d., Novo mesto, Slovenija

Farmacevtski trg v državah srednje in vzhodne Evrope narašča, to pa je priložnost tudi za tuje družbe, da na osnovi notranje in/ali zunanje rasti povečajo prodajo. Notranja oziroma organska rast omogoča povečanje obsega poslovanja s pomočjo naložb v širjenje lastnih proizvodnih in prodajnih aktivnosti, predvsem na osnovi lastnih razvojno-raziskovalnih dosežkov. Medtem, ko je notranja rast dolgoročen proces, pa je zunanja rast praviloma hitrejša in bolj učinkovita, saj na ta način optimalno izkoristimo že obstoječe vire. V kategorijo zunanje rasti prištevamo nekapitalske in kapitalske povezave, med katerimi so največkrat omenjeni prevzemi. Tuje inovativne in generične družbe so v večini držav srednje in vzhodne Evrope kapitalsko že prisotne, tako z lastnimi proizvodnimi obrati kot z večinskimi deleži v domačih družbah. Lahko rečemo, da je bila konec avgusta 2002 edino slovenska farmacevtska industrija v večinski domači lasti. Tuje družbe so torej že prevzele večji del domačih farmacevtskih družb ter raziskovalne inštitute, vendar je še nekaj možnosti za strateška partnerstva in akvizicije predvsem že obstoječih domicilnih družb, pa tudi novih, hitro rastočih in uspešnih manjših družb, proizvajalcev s specifičnimi oziroma nišnimi proizvodi ter distributerskih družb. Možnosti za rast pa obstajajo tudi v sodelovanju s tujimi ali domicilnimi družbami pri ustanavljanju skupnih naložb kot tudi pri posameznih projektih.

Internationalization of Pharmaceutical Companies in East Europe

D. Šuštaršič
Faculty of Economics and Business, University of Maribor, Slovenia
Supervisor: Z. Bajc
Krka, d. d., Novo mesto, Slovenia

Pharmaceutical market in Central and Eastern Europe is growing up and this is an opportunity for foreign companies to use internal and/or external growth to increase their sale. Internal growth means investment in own production and sale capacities based especially on own research and development. While internal growth gives results in relatively longer period, external growth is faster and more effective because existed resources and capacity can be used. External growth comprises strategic coalitions and capital investment. Foreign innovative and generic pharmaceutical industry is already present in Central and Eastern Europe with own production and sale departments as well as majority owners of domestic companies. At the end of august 2002 only Slovenian pharmaceutical industry had majority domestic ownership. Irrespective of aforesaid there are still opportunities for strategic coalitions and takeovers of existing domestic companies, faster growing-up new perspective companies, niche and distribution companies. Pharmaceutical companies could also co-operate in joint ventures and in specific projects.

Vpliv spolnih hormonov na nastanek akutnih ishemičnih poškodb pri izoliranem podganjem srcu

J. Šuštaršič
Medicinska fakulteta, Univerza v Ljubljani, Slovenija
Mentorica: M. V. Budihna
Somentor: G. Drevenšek
Medicinska fakulteta, Univerza v Ljubljani, Slovenija

Spol je pomemben dejavnik pri pogostnosti nekaterih ishemičnih bolezni srca. V naši nalogi smo želeli ugotoviti vpliv kroničnega dajanja estradiola in testosterona podganam ter vpliv brejosti podgan na nastanek miokardnih okvar, povzročenih z akutno ishemijo/reperfuzijo pri izoliranih srcih teh živali. Poskuse smo izvajali na izoliranih srcih spolno zrelih podgan (230-330g) obeh spolov. Srca podgan smo izolirali po Langendorffu in jih 30 minut perfundirali z raztopino Krebs-Henseleit, nasičeno s karbogenom. Nato smo jih izpostavili 40 minutni globalni ishemiji, čemur je sledila 55 minutna reperfuzija. Za oceno stopnje okvare miokarda, povzročene z ishemijo/reperfuzijo, smo uporabili naslednje merjene spremenljivke: pretok skozi koronarne arterije, stopnjo sproščanja laktatne dehidrogenaze (LDH), tlak v levem prekatu, frekvenco utripov srca in trajanje prekatnih fibrilacij v času reperfuzije. Postishemični koronarni pretok je bil višji pri srcih podgan, ki smo jim dajali estradiol oz. testosteron kot pri kontrolnih skupinah. Pri srcih samic z dodanim estradiolom je bil koronarni pretok značilno višji ($p < 0,05$) kot pri srcih kontrolne skupine samic. Najvišje vrednosti sproščanja LDH smo izmerili pri skupini samic po kotitvi. Frekvenca srčnih utripov je bila po ishemiji nižja kot pred ishemijo, najvišja je bila pri skupini samic po kotitvi. Trajanje fibrilacij je bilo pri skupinah z dodanima hormonoma krajše kot pri kontrolnih skupinah, vendar so bile razlike neznačilne. Razlike v postishemičnih spremembah merjenih spremenljivk med poskusnimi skupinami kažejo na zaščitno vlogo spolnih hormonov pri nastanku okvar na izoliranih podganjih srcih med ishemijo/reperfuzijo.

The Influence of Sex Hormones on the Development of Acute Ischaemic Injury of Isolated Rat Heart

J. Šuštaršič
Faculty of Medicine, University in Ljubljana, Slovenia
Supervisor: M. V. Budihna
Co-supervisor: G. Drevenšek
Faculty of Medicine, University in Ljubljana, Slovenia

Sex might be an important factor in the development of some ischaemic diseases of the heart. In our study we tried to assess the influence of subchronical treatment of rats with oestradiol or testosterone and the influence of gravidity on the development of myocardial damage caused by ischaemia/reperfusion. Experiments were carried out on the isolated hearts of sexually mature rats of both sexes (230-330g). We isolated rat hearts according to Langendorff's method. The hearts were perfused with Krebs-Henseleit solution aerated with carbogen for 30 min and then subjected to 40 min zero-flow ischaemia followed by 55 min reperfusion. The severity of myocardial damage caused by ischaemia/reperfusion was assessed by postischaemic changes in the following registered parameters: coronary flow, lactate dehydrogenase release rate (LDH), left ventricular pressure (LVP), heart rate and duration of ventricular fibrillation during reperfusion. The coronary flow was higher in the hearts of rats treated with oestradiol or testosterone than in the hearts of control groups. In the hearts of rats treated with oestradiol the postischaemic coronary flow was significantly ($p < 0.05$) higher than in the hearts of control female group. The highest postischaemic LDH values were observed in the postgravid group. Postischaemic heart rate values were lower than before ischaemia. The highest values were observed in the postgravid group. The duration of postischaemic ventricular fibrillation was shorter in the hormone-pretreated groups. In our experiment a protective role of sex hormones on development of ischaemic/reperfusion damage in isolated rat hearts has been shown.

Genetska heterogenost virusnih sevov bovine virusne diareje (BVD), izoliranih v Sloveniji

I. Toplak
Veterinarska fakulteta, Univerza v Ljubljani, Slovenija
Mentor: J. Grom
Somentorica: D. Barlič-Maganja
Veterinarska fakulteta, Univerza v Ljubljani, Slovenija

Od leta 1997-2001 smo v okviru preiskav za nadzor in izkoreninjenje virusa bovine virusne diareje v Slovenji izolirali štiriinštirideset virusov iz štiriindvajsetih rej v štirih različnih regijah Slovenije. V večini primerov smo viruse BVD dokazali pri perzistentno okuženih živalih in jih analizirali na molekularni ravni. Vsi izolati virusov BVD so bili necitopatogeni. Iz inficirane celične kulture smo izolirali virusno RNA, izvedli reverzno transkripcijo in odsek virusnega genoma v 5'NCR pomnožili z metodo PCR s specifičnimi začetnimi oligonukleotidi. Po pomnoževanju smo produkt PCR prečistili in ga sekvenirali. Na podlagi filogenetske primerjave dobljenih sekvenc smo vseh štiriinštirideset izolatov uvrstili v genotip BVD 1, virusov genotipa BVD 2 nismo našli. Glede na razdelitev virusov BVD smo naše goveje izolate virusov BVD uvrstili v podtip BVD 1f (n = 21), podtip BVD 1d (n = 17), podtip BVD 1b (n = 4), podtip BVD 1g (n = 1), virus, izoliran pri prašiču, pa v podtip BVD 1a. Podobne podtipe virusov BVD so našli v sosednjih evropskih državah. S primerjavo sekvenc v 5'NCR smo ugotovili, da smo izolirali najmanj 14 različnih sevov. Sekvence naših izolatov se razlikujejo od tistih, ki so zajeti v genomski banki podatkov (GenBank). Virusi, ki smo jih izolirali pri živalih v isti reji, so bili z izjemo ene reje enaki. Razlike v sekvencah, ki smo jih ugotovili pri 14 sevih virusov BVD, so bile na področju variabilnih regij V2 in V3. V regiji V3, na mestu 300-306 glede na referenčni sev virusa BVD NADL, smo pri 12 izolatih virusov BVD našli delecijo petih nukleotidov. Kljub tej deleciji sta predvideni sekundarni strukturi 5'NCR v dolžini 216 nukleotidov našega izolata virusa BVD 1085/00-B (BVD 1f) in referenčnega seva virusa BVD Oregon C24V (BVD 1a) enaki.

Genetic Heterogeneity of Bovine Viral Diarrhoea Virus (BVDV) Strains Isolated in Slovenia

I. Toplak
Veterinary Faculty, University of Ljubljana, Slovenia
Supervisor: J. Grom
Co-supervisor: D. Barlič-Maganja
Veterinary Faculty, University of Ljubljana, Slovenia

In the frame of Slovenian bovine viral diarrhoea virus (BVDV) control and eradication programme forty-four viruses isolated during 1997-2001 mainly from persistently infected (PI) animals found in twenty-four different cattle herds from four regions of Slovenia were analysed on the molecular level. Viruses were propagated on cell culture and were found to be of noncytopathogenic biotype. Viral RNA was extracted from infected cell cultures, reversely transcribed and amplified by PCR with primers targeting the 5'NCR, followed by direct sequencing of purified PCR product. Using phylogenetic analysis, all forty-four isolates were classified into BVDV type 1, while no BVDV type 2 were found. According to the nomenclature used to distinguish BVDV, Slovenian bovine isolates were of the subtype BVDV 1f (n = 21), BVDV 1d (n = 17), BVDV 1b (n = 4), BVDV 1g (n = 1), whilst the porcine isolate was of subtype BVD 1a. This genetic prevalence matched those previously reported for neighbouring European countries. The alignment of the 5'NCR sequences of all Slovenian isolates showed that several of them were identical, giving a total number of 14 different sequences. None of the obtained 5'NCR sequences were identical to the sequences previously submitted to the GenBank. From eight cattle herds several BVDV isolates were analysed; with one exception all the isolates from each herd were of the same genetic group. Between 14 sequences most of the mutational differences were located in two variable regions, previously termed regions V2 and V3. In 12 BVDV isolates, a deletion of five nucleotides in the region V3 of 5'NCR was found, which corresponds to nucleotides 300-306 in the reference strain BVDV NADL. Despite these deletions, the predicted secondary structures of 216 nucleotide long 5'NCR fragment of the isolate BVDV 1085/00-B (BVDV 1f) and the reference strain BVDV Oregon C24V strain (BVDV 1a) were identical.

Določanje nekaterih anionov in organskih kislin s kapilarno elektroforezo

R. Toporišič
Fakulteta za kemijo in kemijsko tehnologijo, Univerza v Ljubljani, Slovenija
Mentor: B. Pihlar
Fakulteta za kemijo in kemijsko tehnologijo, Univerza v Ljubljani, Slovenija

S kapilarno elektroforezo lahko določimo širok spekter spojin. Nabite delce ločujemo s kapilarno consko elektroforezo, nevtralne pa z micelarno elektrokinetično kromatografijo. Ločljivost in učinkovitost ločbe lahko povečamo tako, da spreminjamo delovno napetost, koncentracijo pufra, temperaturo, pH pufra ali pa z dodatkom organskega topila. Če določevana spojina slabo oz. ne absorbira v UV področju, lahko ta problem rešimo tako, da uporabimo indirektno detekcijo. Ko se eluira naša komponenta, dobimo na elektroferogramu negativen vrh. Pri določanju majhnih anionov skrajšamo čas analize tako, da z dodatkom kationske površinsko aktivne snovi spremenimo naboj notranje stene kapilare. Z micelarno elektrokinetično kromatografijo pa lahko z dodatkom ciklodekstrinov ločimo tudi optične izomere. Pred vsako meritvijo ni potrebno menjavati pufer na anodni strani. Če pa opazujemo migracijske čase, je potrebno pred vsako meritvijo zamenjati pufer in se tako izogniti elektrolizi pufra. Pri določanju koncentracije merjenih spojin z umeritveno krivuljo in s standardnim dodatkom dobimo z upoštevanjem eksperimentalne napake primerljive rezultate.

Determination of some Anions and Organic Acids by Capillary Electrophoresis

R. Toporišič
Faculty of Chemistry and Chemical Technology, University of Ljubljana, Slovenia
Supervisor: B. Pihlar
Faculty of Chemistry and Chemical Technology, University of Ljubljana, Slovenia

Several compounds can be determined by electromigration techniques. Capillary zone electrophoresis is used for separation of charged analytes and micellar electrokinetic chromatography is suitable for separation of neutral compounds. Separation efficiency can be boosted by altering voltage, concentration and pH of buffer, temperature or by adding organic solvent. Indirect detection is used when compound under investigation does not absorb UV light. A negative peak is obtained in electropherogram, when the compound is eluted. By adding cationic surfactant, the charge on inner capillary wall is altered and consenquently time of analysis of smaller anions is shortened. The addition of cyclodextins in micellar electrokinetic chromatography can result in separation of optical isomers. Replacing buffer before each measurment on the anode side is not necessary. On the other hand, when observing time of migration, buffer must be replaced after each run to prevent electrolysis of buffer. Concentration can be determined by calibration curve or standard addition. Both methods yield comparable results.

Zanesljivost lestvice obsega gibljivosti "Pediatric Escola Paulista de Medicina Range of Motion"

V. Vrbinc
Visoka šola za zdravstvo, Univerza v Ljubljani, Slovenija
Mentor: M. Jakovljević
Visoka šola za zdravstvo, Univerza v Ljubljani, Slovenija
Somentorica: M. Kuhar
Univerzitetni klinični center, Ljubljana, Slovenija

Uvod: za merjenje funkcijskega statusa otrok z juvenilnim idiopatskim artritisom (JIA) se uporabljajo različna orodja, vendar nobeno ne temelji na vrednotenju obsega giba. V nalogi je predstavljena Otroška EPM-ROM lestvica, ki temelji na merjenju aktivno izvedenih desetih obsegov gibov in so jo izdelali v Braziliji. Metode dela: 7 otrok z JIA je bilo testiranih na Pediatrični kliniki (Služba za alergologijo in revmatske bolezni), 13 otrok pa na obnovitveni rehabilitaciji v Dolenjskih Toplicah. Testiranje je potekalo v jutranjih urah (7.30 - 8.30) v razmiku dveh dni. Testiranje je potekalo po metodi "test-ponovni test" z enim preiskovalcem. Rezultati: povprečje Otroške EPM-ROM lestvice je bilo 0,76 (SD ± 0,53). Pearsonov koeficient korelacije za prvo in drugo meritev za izmerjene obsege gibov v kotnih stopinjah je bil r= 0,9325 (p<0,001). Pearsonov koeficient korelacije za povprečno oceno obsegov gibov je bil r= 0,9992 (p<0,001). Spearmanov koeficient korelacije obsegov gibov po Otroški EPM-ROM lestvici (z ocenami od 0 do 3) je bil rs=0,9303 (p<0,01) in za vsoto obsegov giba rs=0,9970 (p<0,01). Zaključek: zanesljivost slovenskega prevoda Otroške EPM-ROM lestvice je zelo visoka. Otroška EPM-ROM lestvica je preprosto orodje, ki ne zahteva veliko finančnih sredstev in za katerega izvedbo ne potrebujemo veliko časa.

Evaluation of Reliability of "Pediatric Escola Paulista de Medicina Range of Motion"

V. Vrbinc
University College of Health Care, University of Ljubljana, Slovenia
Supervisor: M. Jakovljević
University College of Health Care, University of Ljubljana, Slovenia
Co-supervisor: M. Kuhar
University Clinical Center, Ljubljana, Slovenia

Objective: functional status of children with juvenile idiopathic arthritis is measured by using different instruments. None of them is developed on range of motion evaluation. In the work is introduced Pediatric EPM-ROM Scale (developed in Brazil) which is developed on the basis of measurement of ten active performed ranges of motion. Methods: 7 children were tested on Department of Pediatrics (Division of Allergology, Rheumatology and Clinical Immunology) and the other (13) at the reconstitution rehabilitation in Dolenjske Toplice. The testing was performed 2 days apart, always in the morning (between 7.30 and 8.30) on the method test-retest with one observer. Results: average value of Pediatric EPM-ROM Scale was 0.76 (SD ± 0,53). The Pearson correlation coefficient comparing first and second measurement of range of motion (in degrees) was r= 0.9325 (p<0.001); the Pearson correlation coefficient for comparison of first and second measurement of average range of motion's values was r= 0.9992 (p<0,001). The Spearman correlation coefficient comparing range of motion in Pediatric EPM-ROM Scale (values 0-3) was rs= 0.9303 (p<0,01) and for the sum of range of motion rs= 0.9970 (p<0,01). Conclusion: reliability of Slovene translation of Pediatric EPM-ROM Scale was very high. It is very simple instrument that does not require high finances and does not take long time for its performance.

Statins in the Therapy of Hyperlipoproteinemias and Coronary Heart Disease

G. Zdunić
Faculty of Pharmacy, Belgrade, FR Yugoslavia
Supervisor: N. Ugrešić
Faculty of Pharmacy, Belgrade, FR Yugoslavia

Hyperlipoproteinemias are the major cause of atherosclerosis and atherosclerosis-associated diseases, such as coronary heart disease (CHD), ischemic cerebrovascular disease, and peripheral vascular disease. Recognition of hyperlipoproteinemias as a risk factor has led to the development of drugs that reduce cholesterol levels. Among these, the statins are the most effective and well-tolerated agents. These drugs are competitive inhibitors of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, which catalyzes an early, rate limiting step in endogenous cholesterol biosynthesis.
Statins lower low-density lipoproteins cholesterol (LDL-C) by 20% to 55%, depending on the dose and compound used. The influence on the other parameters of lipid status (triglycerides and HDL-C) is less predictable and highly dependent on the basal level and the statin used. Other potentially clinically relevant effects include stabilizing endothelial function, suggested antiinflammatory effects, reducing the susceptibility of lipoproteins to oxidation, as well as protective effects on plaque stability. These effects may contribute to the cardioprotective action established in a number of well-controlled clinical trials. These studies documented efficacy and safety of statins in reducing fatal and nonfatal CHD events and lowering total mortality. Accordingly, statins are becoming an evidence-based part of contemporary cardiovascular diseases treatment.

Šaržna rektifikacija zmesi voda - N-metilimidazol

L. Zupančič
Fakulteta za kemijo in kemijsko tehnologijo, Univerza v Ljubljani, Slovenija
Mentor: J. Golob
Fakulteta za kemijo in kemijsko tehnologijo, Univerza v Ljubljani, Slovenija

N-metilimidazol uporabljajo v sintezi različnih organskih materialov kot katalizator pri nekaterih reakcijah aciliranja, v nekaterih primerih pa služi tudi kot topilo. Pri uporabi večjih količin ga je zaradi ekoloških zahtev in visoke cene smiselno regenerirati in vračati v sintezni postopek.
V tem delu je nakazana metoda za regeneracijo N-metilimidazola iz sistema voda - N-metilimidazol. V ta namen so bili pridobljeni ravnotežni podatki tekoče-para za sistem voda - N-metilimidazol. Refraktometrija se je izkazala kot ustrezna fizikalno-kemijska metoda za določanje sestave zmesi, kar je zaradi enostavnosti meritev zelo pomembno tudi za morebitno industrijsko aplikacijo. Dobljeni rezultati lahko služijo kot izhodišče za načrtovanje naprave za regeneracijo N-metilimidazola v industrijskem merilu.

Batch Distillation Of Water - N-methylimidazole System

L. Zupančič
Faculty of Chemistry and Chemical Technology, University of Ljubljana, Slovenia
Supervisor: J. Golob
Faculty of Chemistry and Chemical Technology, University of Ljubljana, Slovenia

N-Methylimidazole is used as a catalyst in the synthesis of various organic materials and in some cases as a solvent. In the industrial processes where the large quantities of N-methylimidazole are used the need for the regeneration and recycling is obvious because of its high price and ecological requirements.
In this work a method for solvent recovery from water - N-methylimidazole system is proposed. For this purpose the vapour-liquid equilibrium diagram was determined and refractometry was used as a convenient physico-chemical method for the determination of water content in the mixture.
Results of this work can be used for design of an apparatus for the regeneration of N-methylimidazole in industrial scale.

Barve cvetlic - naravni indikatorji?

M. Car, P. Legan, M. Turk
Gimnazija Novo mesto, Slovenija
Mentorica: M. Kovač
Gimnazija Novo mesto, Slovenija

V našem raziskovalnem delu smo pridobivali ekstrakte barvil iz cvetov. Te ekstrakte smo nato podvrgli različnim vplivom: kisline, baze oksidanta in reducenta in opazovali, kako se barva ekstrakta spremeni. Sklepali smo, da so barve cvetov močno odvisne od različnih dejavnikov okolja (različni plini v ozračju, topne snovi v tleh, pH…). Želeli smo tudi dobiti odgovor, zakaj je največ rumenih rastlin in ali so vse rumene barve iz istih barvnih komponent (zato smo izvedli papirno kromatografijo). Poskusili smo se tudi v barvanju bombažnih krpic ter pri tem preučevali učinke različnih čimž na intenzivnost barv na krpicah.

Colors of the Flowers - Natural Indicators?

M. Car, P. Legan, M. Turk
Gimnazija Novo mesto, Slovenia
Supervisor: M. Kovač
Gimnazija Novo mesto, Slovenia

In our research work we prepared extracts of colors from flowers. After preparation, we underwent these extracts to different influences: acid, base, oxidant and reductant, and observed, how the color of the extract changes. We assumed that colors of the flowers very much depended on various factors of the environment (different gases in the atmosphere, soluble substances in the ground, pH…). We also wanted to find out why there are so many yellow plants, and if all yellow colors are from the same coloring components (that's why we conducted the paper chromatography). We tried ourselves in dyeing small cotton pieces of cloth as well, and studied the effects of different molders on the intenseness of colors on these cloth pieces.

Napitki športnikov

N. Murko, B. Turk, M. Rangus
Gimnazija Novo mesto, Slovenija
Mentorica: K. Kikelj
Krka, d. d., Novo mesto, Slovenija

Naloga je razdeljena na teoretični del, v katerem razpravljamo o potrebah športnikov, vitaminih, mineralih in napitkih, ter v praktični del, v katerem so na osnovi rezultatov ankete po preizkusu napitka podani zaključki.
Večjo potrebo po vitaminih in mineralih imajo gotovo športniki, saj med vadbo porabijo večjo količino vode in elektrolitov, ki jih morajo čimprej pridobiti nazaj. Z znojenjem se zgubljajo znatne količine mineralnih soli predvsem K, Mg, Na in Ca. Te snovi pa nadomestimo tako, da med in po vadbi uživamo napitke z dodatki mineralov.
Za vsakega športnika pa je tudi značilna povečana zahteva po vitaminih. To potrebo pa povečajo še določeni dejavniki, ki pa so povezani s športom (sprememba, nadmorska višina, sprememba klimatskih in geografskih prostorov, previsoka ali prenizka temperatura, itd..).
Če športnik ne zaužije dovolj izgubljenih snovi, pa lahko sledijo nepričakovani zapleti in poškodbe, zato se je dobro posvetiti izotoničnim napitkom, ki nam s konstantnim uživanjem preprečijo nepotrebne težave.
V analizi sta opisani dve anketi, ki smo ju uporabljali. Anketa, ki je bila namenjena športnikom, je vsebovala vprašanja o Vitanova Izomaratonik granulatu (zraven ankete smo priložili tudi vrečke Vitanova Izomaratonika). Splošna anketa pa je vsebovala vprašanja o napitkah oziroma o poznavanju napitkov med ljudmi, kaj je ključnega pomena, da bi jih kupili, kaj vpliva na njihovo odločitev glede nakupa, koliko napitkov poznajo... Vključile smo tudi vprašanja o Krkinem izdelku Vitanova Izomaratonik, in sicer koliko ga ljudje poznajo, kaj menijo o njegovem okusu in če bi ga uporabljali.

Sport Beverages

N. Murko, B. Turk, M. Rangus
Gimnazija Novo mesto, Slovenia
Supervisor: K. Kikelj
Krka, d. d., Novo mesto, Slovenia

Project is devided on theoretical part, which includes discussion about needs of sportsmens, vitamins, minerals and special tonics. Practical part is based on quesstionare results after tastuing isotonic beverage.
Sportsmen have larger need for vitamins and minerals. During the exercise a big quantity of water and electrolytes is lost, which have to be replaced as soon as possible. A lot of K, Mg, Na and Ca salts are lost in the perspiration. To replace those substances, we drink special tonic during and after exercise.
Larger need for vitamins is also characteristic for every sportsman. This need is enlarged by some factors connected to sports (quick change, altitude, change of climatic or geographic characteristic, too low or too high temperature ...)
If sportsman does not use/replace enough of the lost substances, some unexpected problems and injuries may appear. Therefore it is good to dedicate some time to isotonic drinks, because with constant use they can prevent unnecessary problems.
In analysis two questionnaires are described. Questionnaire, meant for sportsmen, is about Krka's product Vitanova Izomaratonik (we add a sample of it, for easier estimation). Other questionnaire contains questions about drinks, how well people know them, what is important to know before purchase, what influences their decision, how many drinks they know and some questions about Vitanova Izomaratonik (how well they are informed, use, taste,…)

Kloniranje humane topoizomeraze II α

K. Plaper, K. Turk
Gimnazija Novo mesto, Slovenija
Mentorica: A. Plaper
Krka, d. d., Novo mesto, Slovenija
Somentorica: N. Petakovič
Gimnazija Novo mesto, Slovenija

Humana topoizomeraza II α je encim, ki je v celicah odgovoren za spreminjanje topološke oblike DNK. V nalogi smo se ukvarjali s kloniranjem cDNK humane topoizomeraze II α (hTOPIIα). Uporabili smo osnovne tehnike molekularne biologije. cDNK hTOPIIα smo uspešno namnožili z verižno reakcijo polimeraze (PCR). Kot osnovo za pomnoževanje smo izbrali predhodno pripravljen plazmid YEp WOB6. Uporabili smo začetne oligonukleotide, YEP in TOP-T, s pomočjo katerih smo v cDNA vnesli nova restrikcijska mesta. Ta omogočajo prestavljanje cDNA v ugoden ekspresijski vektor in pridobivanje večje količine hTOPIIα encima. Za pomnoževanje cDNK smo uporabili encim elongazo, ki je mešanica dveh encimov in je primerna za pomnoževanje daljših matričnih DNK, zagotavlja pa tudi proces samopopravljanja. Namnožen produkt smo analizirali z agarozno elektroforezo. Ugotovili smo, da smo pridobili zadovoljivo količino produkta PCR primerne velikosti. Produkt, cDNK hTOPIIα, smo izrezali iz gela, očistili in ga vključili v vektor TOPO XL, ki je oblikovan tako, da je primeren za direktno kloniranje produktov PCR in nosi selekcijski marker za kanamicin. Po reakciji spajanja cDNK z vektorjem smo produkte prenesli v kompetentne bakterije, v katerih se je vektor množil in s tem proizvajal številne kopije samega sebe in gena, ki ga je nosil, cDNA hTOPIIα. Bakterije smo razmazali na selektivno gojišče z antibiotikom kanamicinom, ki zagotavlja rast tistih bakterij, ki vsebujejo rekombinantni plazmid, z genom za rezistenco na antibiotik kanamicin. Na ploščah je zraslo 69 kolonij. Izbrali smo 14 kolonij za analizo klonirane cDNK. Iz tekočih kultur smo izolirali plazmidno DNK. Restrikcijska analiza DNK posameznih klonov z encimi Xho I, Not I in Eco RI je pokazala, da eden izmed klonov vsebuje cDNK humane topoizomeraze II α. S pomočjo rekombinantne tehnologije smo uspešno prenesli cDNK humane topoizomeraze II α v vektor TOPO XL. Klon je osnova za nadaljnje raziskave v Krkinih laboratorijih.

Human Topoisomerase II α Cloning

K. Plaper, K. Turk
Gimnazija Novo mesto, Slovenia
Supervisor: A. Plaper
Krka, d. d., Novo mesto, Slovenia
Co-supervisor: N. Petakovič
Gimnazija Novo mesto, Slovenia

Human topoisomerase II a (hTOPIIα) is an enzyme which catalyses topological DNA changes in the cell needed for the basic living procedures. In our research the basic techniques of molecular biology were used for hTOPIIα cDNA cloning. hTOPIIα cDNA was amplified with polymerase chain reaction (PCR). Preliminary prepared YEp WOB6 plasmid was chosen as a template for cDNA amplification. Primers YEP and TOP-T introduced new restriction sites to cDNA's ends, enabling the transfer of the cDNA into suitable expression vector and production of large amounts of hTOPIIα enzyme. For cDNA amplification elongase enzyme mix was used. The system contains a mixture of two enzymes and it is appropriate for longer DNA templates amplification since it also contains a proofreading enzyme. The amplified product was analysed with agarose gel electrophoresis. The analysis indicated that sufficient amount of PCR product of proper size was produced. hTOPIIα cDNA was cut out from the gel, purified, and transfered into TOPO XL vector with canamycin selection marker, suitable for direct PCR products cloning. After cDNA combined with the vector TOPO XL, the appropriate competent bacterial cells were transformed with recombinant product. Transformed bacterial cells were plated on the selective agar plates with antibiotic kanamicyn allowing the growth of the colonies containing a recombinant plasmid with kanamycin selection gene. 69 colonies were identified on the plates. We chose 14 colonies for analysis of cloned cDNA. Plasmid DNA was isolated from the liquid cultures. The restriction analysis of individual DNA clones with Xho I, Not I and Eco RI indicated that one of the clones held hTOPIIα cDNA. By using the recombinant technology we successfully transferred hTOPIIα cDNA into TOPO XL vector. The clone is a base for further research in Krka's laboratories.

Seznam avtorjev / Author Index

Seznam mentorjev / Supervisor Index

Uredniški odbor

Natalija Vitezić, glavna urednica
Miha Japelj
Mateja Vukšinič Kozoglav
Alenka Štefanič Petek

Oblikovanje

Miran Klenovšek, Gigodesign, Ljubljana

Produkcija

EFOR, d.o.o., Medvode

Tisk

Kočevski tisk, Kočevje

Izdajatelj

KRKA, d. d., Novo mesto, Slovenija

www.krka.si/krkine-nagrade
www.krka.si/krka-prizes