KRKA

33. Krkine nagrade
33rd Krka Prizes

Novo mesto, Slovenija, 23.-24. oktober 2003

13. mednarodni simpozij
13th International Symposium

33

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

Častni odbor 33. 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
Ivan Rozman, rektor Univerze v Mariboru
Matjaž Jeras, predsednik 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 Kralj-Pučko
Ivan Radež
Janja Požar
Dušan Dular
Aleš Hvala
Jože Gnidovec
Irena Čarman
Damjan Možina

Znanstveni odbor 13. mednarodnega simpozija

Miha Japelj, predsednik
Irena Čarman
Aleš Hvala

Recenzenti

Milan Banovec, Neda Benički-Švagelj, Pija Berus, Tadeja Bevec, Renata Bibič, Marko Boh, Marjanca Breznik, Matej Breznik, Nina Cimerman, Lea Cvitkovič-Maričič, Irena Čarman, Jože Drinovec, Branislav Filipović, Vesna Flego, Mihael Florjanič, Aleš Gasparič, Jože Gnidovec, Mojca Golob, Nataša Gornik, Marija Grčman, Barbara Guštin, Pavica Hajko, Aleš Hvala, Marijan Ivanuša, Miha Japelj, Eva Jožef, Brane Kastelec, Milena Kastelic, Ksenija Kikelj, Saša Kolarič, Janko Kos, Berta Kotar-Jordan, Helena Kotnik, Pavel Kovač, Zdenka Kranjc-Gregorčič, Milka Križman-Pečar, Andreja Kuhar, Borut Lekše, Roman Lenaršič, Jože Malenšek, Mateja Merlin, Marjo Merslavič, Mateja Merslavič, Darinka Miklavčič, Damjan Možina, Andreja Novak, Vesna Pahor, Urška Papež, Smiljan Pavišič, Viljem Pavli, Diana Pavlič-Pokorny, Tatjana Pečnik, Mitja Pelko, Stanka Perc, Robert Pišek, Andreja Plaper, Igor Plaper, Nadja Počrvina, Vladimir Pokorny, Janja Požar, Sandra Pišek, Aleša Punčuh-Kolar, Ivan Radež, Anton Recelj, Monika Reflak, Aleš Rotar, Miloš Ružič, Mirjam Milharčič-Simčič, Igor Simonič, Gordan Sladič, Janez Smodiš, Jasna Strel, Leon Ščuka, Polona Šolmajer-Lampič, Mojca Štaudohar-Kozjan, Božena Šuštar, Jaroslav Tihi, Robert Ucman, Anamarija Vajs, Nada Vinder, Natalija Vitezić, Tomaž Vrbinc, Franc Vrečer, Stane Zalar, Janez Zupančič, Silvo Zupančič, Slavko Zupančič, Damjana Zupančič-Božič, Rok Zupet

Vsebina

33. Krkine nagrade / Krka Prizes

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

33. Krkine nagrade

33rd Krka Prizes

Tu smo, rastemo in naprej gremo

V preteklem ducatu let, skozi katere je vijugalo naše življenje, se je spremenilo veliko. Morda več, kot smo si lahko zamislili za vse svoje življenje. Tako teorije kakor dejansko dogajanje so korenito zamajali občutek samozadostnosti in določenosti v družbi, zlasti pa v znanosti. Vsemu navkljub ali prav zato smo preživeli in postali boljši. To velja za vsakogar od nas, seveda na drugačen način in po svojevrstni, neponovljivi poti. Ena od stalnic tekom neprestanega spreminjanja je učenje. Ta neoprijemljivi, a nepogrešljivi in povsod prisotni fenomen, ki nam je omogočil prilagajanje, je tudi edina smernica na nadaljnji poti. Vsak od mladih raziskovalcev, vsak od mentorjev, tudi vsakdo v družini, razredu, okolju… je prispeval svoje k neponovljivemu mozaiku idej, dela in dosežkov, ki so vtkani v letošnje naloge, nagrajene s Krkinimi nagradami. Vsak od kamenčkov je biser zase, celotna slika pa hrabri in spodbuja: tu smo, rastemo, naprej gremo.

We are Here, We are Growing and Making Progress

The past dozen of years, through which our life has twisted and turned, are characterized by many changes - maybe more than we could imagine for the whole of our lives. Both theories and actual happening have radically shaken the feeling of self-sufficiency and specificity in society, particularly in science. Despite everything or, perhaps, because of it, we have survived and even grown better. This is true for each one of us – in a different way and in an original, unrepeatable manner, of course. One of the constants throughout the continuous changing is learning. This abstract but indispensable and commonly present phenomenon, which has enabled us to adapt, is the only guideline in our further way. Each of the young researchers, each of the mentors, family, class, environment, etc. has contributed their portion to the unique mosaic of ideas, work and achievements that are interlaced in this year's papers receiving Krka Prizes. Each of the small mosaic stones is a pearl in itself, but the whole design encourages and stimulates: we are here, we are growing and making progress.

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 2003 / Krka Prize Winners 2003

Št.	Nagrajenci	Status	Mentor, Somentor(ji)
1838	JURE AČIMOVIČ	študent medicine	Martina FINK Damjana ROZMAN
1839	SONJA BARIČ	študentka farmacije	Milica MURN Marija SOLLNER DOLENC
1840	ALJOŠA BAVEC	podiplomski študent medicine	Matjaž ZORKO
1841	DAVID BELE	dijak	Jaroslav TIHI
1842	TEJA BREZOVAR	dijakinja	Marinka KOVAČ Martina STRMOLE
1843	MAJA BREŽE	študentka farmacije	Marija SOLLNER DOLENC Marko ANDERLUH
1844	MIRKO CEVEC	študent kemije	Matjaž POLAK Janez PLAVEC
1845	MARKO COTMAN	doktorand	Damjana ROZMAN Gregor MAJDIČ
1846	PETER CVELBAR	študent kemije	Nataša BUKOVEC Sabina GRABNER
1847	ANDREJA ČANŽEK-MAJHENIČ	doktorandka	Irena ROGELJ Darja ŽGUR-BERTOK
1848	IRENA ČARMAN	doktorandka	Peter RASPOR Margareta GLANCER-ŠOLJAN
1849	PETRA ČEBAŠEK	študentka kemije	Jurij SVETE
1850	URŠKA ČEGOVNIK	doktorandka	Srdjan NOVAKOVIĆ
1851	TADEJ ČEPELJNIK	študent Biotehnične fakultete	Romana MARINŠEK-LOGAR
1852	MARTIN ČRNUGELJ	doktorand	Janez PLAVEC
1853	BILJANA DŽAJKOVSKA	podiplomska študentka farmacije	Aleš MRHAR
1854	MARTINA FINK	doktorandka	Damjana ROZMAN
1855	MAJA FRANKO	dijakinja	Vojka ZUPANČIČ Jana GORŠIN-FABJAN
1856	VIDA FRANKOVIČ	študentka kemijske tehnologije	Peter KRAJNC
1857	PETRA HOČEVAR	dijakinja	Marinka KOVAČ Martina STRMOLE
1858	JANJA JAKŠE	študentka kemije	Lucija ZUPANČIČ-KRALJ Nives ŠOŠTARIĆ-VIRC
1859	RENATA JAKŠE	doktorandka	Branko STANOVNIK
1860	EVA JAMBROŠIČ	študentka kemije	Matic LEGIŠA
1861	SAŠA JENKO	študentka biokemije	Dušan TURK Gregor GUNČAR
1862	ZALA JEVNIKAR	študentka farmacije	Borut ŠTRUKELJ Nina SLAPAR
1863	ČEDOMIR JOKSIMOVIĆ	podiplomski študent medicine	Andrej CÖR
1864	IVANA KADIČ	dijakinja	Helena KOTNIK Sonja KERMC Barbara BAJC-ŠEROVIČ
1865	REBEKA KALČIČ	dijakinja	Tatjana DURMIČ Ksenija KIKELJ
1866	LANA KAMBIČ	dijakinja	Marinka KOVAČ Martina STRMOLE
1867	MAJA KINCL	doktorandka	Franc VREČER Marjan VEBER
1868	TINA KOČEVAR	dijakinja	Tatjana DURMIČ Ksenija KIKELJ
1869	BRANKA KOROŠEC	študentka medicine	Metka RAVNIK-GLAVAČ

1870	ANDREJA KOVAČ	študentka farmacije	Stanislav GOBEC Metka FILIPIČ
1871	ALEKSANDRA KRAJNC	študentka Biotehnične fakultete	Nataša DEBELJAK Damjana ROZMAN
1872	HELENA KRALJ	študentka ekonomije	Marjan HRIBAR Maja MAKOVEC-BRENČIČ
1873	TOMAŽ KRALJ	študent farmacije	Robert UCMAN
1874	KATJA KRISTAN	podiplomska študentka medicine	Tea LANIŠNIK-RIŽNER Jure STOJAN
1875	MARIO LAGANOVIĆ	podiplomski študent medicine	Željko REINER
1876	EDGARS LIEPINSH	podiplomski študent kemije	Ruta MUCENIECE
1877	KAJA LJUBEC	študentka farmacije	Marica MEDIĆ-ŠARIĆ
1878	MAJ LUZAR	dijak	Jaroslav TIHI
1879	ANĐELIJA MALENOVIĆ	podiplomska študentka farmacije	Darko IVANOVIĆ Mirjana MEDENICA
1880	TAMARA MILOŠEVIČ-BERLIČ	doktorandka	Peter DOVČ
1881	JANJA NOVOSEL	študentka Filozofske fakultete	Anton GOSAR Marjan ČERNE
1882	NATAŠA OGRAJŠEK	študentka farmacije	Berta KOTAR-JORDAN Franc VREČER
1883	TINA OGRIČ	študentka farmacije	Matjaž JERAS Urška REPNIK
1884	LIDIJA OMAN	študentka farmacije	Mirjana GAŠPERLIN
1885	IRENA OVEN	študentka Biotehnične fakultete	Mojca NARAT
1886	MIHA PAVŠIČ	študent biokemije	Brigita LENARČIČ
1887	MOJCA PLOŠTAJNER	študentka farmacije	Bojan DOLJAK
1888	DARJA PODPEČNIK	podiplomska študentka medicine	Damjan GLAVAČ
1889	FRANCI POGORELC	študent kemije	Ksenija KOGEJ Andrej GARTNER
1890	URŠKA POTOČAR	dijakinja	Vojka ZUPANČIČ Jana GORŠIN-FABJAN
1891	FRANC POŽGAN	doktorand	Marijan KOČEVAR
1892	OJA PRELOVŠEK	študentka medicine	Zoran GRUBIČ Tomaž MARŠ
1893	MATEJA PUCELJ	študentka farmacije	Odon PLANINŠEK Franc VREČER
1894	YULIYA I. RAGINO	podiplomska študentka medicine	Jurij NIKITIN
1895	SIMONA RAJTAR	študentka medicine	Tatjana IRMAN-FLORJANC
1896	MILOŠ REPŠE	dijak	Jaroslav TIHI
1897	JERICA ROZMAN-PUNGERČAR	doktorandka	Vito TURK Boris TURK
1898	POLONA RUDOLF	študentka farmacije	Jože DRINOVEC
1899	LUCIJA RUS	študentka farmacije	Marija BEŠTER-ROGAČ
1900	ANDREJA SIVEC	študentka kemijske tehnologije	Andreja ZUPANČIČ-VALANT
1901	VESNA SLAMERŠEK	študentka farmacije	Saša BAUMGARTNER
1902	GORAZD SOBOČAN	doktorand	Peter GLAVIČ
1903	DAVID ŠARLAH	študent kemije	Andreja MAJERLE Barbara MOHAR
1904	LEON ŠČUKA	doktorand	Peter LAZAR
1905	JAN ŠIMEK	podiplomski študent medicine	Jaromir HRADEC
1906	BOŠTJAN ŠIMUNIČ	doktorand	Vojko VALENČIČ
1907	ŽIVA ŠOŠTER	študentka Ekonomsko poslovne fakultele	Bruno ZAVRŠNIK Sintija VERSTOVŠEK
1908	JERNEJ ŠPILER	podiplomski študent ekonomije	Krešo PUHARIČ
1909	DAMJAN ŠTERK	študent kemije	Barbara MOHAR
1910	MARTIN ŠTIMPFEL	dijak	Vojka ZUPANČIČ Jana GORŠIN-FABJAN

1911	ANDRAŽ ŠUŠTARIČ	študent farmacije	Stane SRČIČ Anemarija ZEGA
1912	JURIJ TRONTELJ	študent farmacije	Marija BOGATAJ
1913	MARTINA TURK	doktorandka	Ana PLEMENITAŠ
1914	DARKO URŠIČ	študent farmacije	Albin KRISTL
1915	JOŽICA VAŠL	študentka kemije	Roman JERALA
1916	TOMAŽ VAUPOTIČ	študent kemije	Vladka ČURIN-ŠERBEC Ruth R. RUPREHT
1917	TANJA VEHOVEC	študentka farmacije	Vojko KMETEC Viljem PAVLI
1918	KATJA VERNIG	študentka farmacije	Joško OSREDKAR
1919	PETRA VERŽUN	študentka farmacije	Marija BOGATAJ
1920	MOJCA VIDMAR-BERUS	podiplomska študentka Ekonomsko poslovne fakultete	Leon REPOVŽ Milan JURŠE Brane KASTELEC
1921	IGOR VIRANT	študent farmacije	Tatjana MATEOVIĆ
1922	SUZANA VOZELJ	študentka farmacije	Aleš MLINARIČ Alberto MORALES VARGAS
1923	IVO VESELIČ	dijak	Helena KOTNIK Sonja KERMC Barbara BAJC-ŠEROVIČ
1924	JERNEJ WAGGER	študent farmacije	Branko STANOVNIK Jurij SVETE
1925	JASNA ZADNIK	dijakinja	Helena KOTNIK Sonja KERMC Barbara BAJC-ŠEROVIČ
1926	RENATA ZAJC	podiplomska študentka kemijske tehnologije	Miha JAPELJ Marjan VEBER
1927	VALENTINA ZAVAŠNIK-BERGANT	doktorandka	Janko KOS
1928	SAMO ZAVRL	študent farmacije	Tatjana MATEOVIĆ
1929	KATJA ZORC	dijakinja	Marinka KOVAČ Martina STRMOLE
1930	ANDREJA ZORKO	študentka Biotehnične fakultete	Mihael J. TOMAN
1931	VOJKO ZUPANČIČ	študent kemije	Lucija ZUPANČIČ-KRALJ
1932	KATJA ŽMITEK	študentka kemije	Marko ZUPAN Jernej ISKRA

33.
Krkine
nagrade
33rd
Krka
Prizes

13. mednarodni simpozij
13th International Symposium
Zbornik povzetkov
Book of Abstracts

13. mednarodni simpozij ob 33. Krkinih nagradah

Krkine nagrade so posvečene predvsem spodbujanju mladih študentov k poglobljenemu študiju, znanju in spoznavanju pomembnih znanstvenih, razvojnih in raziskovalnih dosežkov, ki so ključnega pomena za smotrni razvoj sodobne farmacevtske industrije.
Svečane podelitve Krkinih nagrad postajajo posebna in enkratna 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.
Že pred trinajstimi leti smo začeli povezovati Krkine nagrade z mednarodnimi simpoziji. V našo sredino radi vabimo vrhunske znanstvenike, uveljavljene univerzitetne profesorje, strokovnjake in raziskovalce s celega sveta. Do sedaj smo v Krko povabili že nad 90 uveljavljenih vrhunskih univerzitetnih profesorjev, akademikov in strokovnjakov (med drugim tudi Nobelovega nagrajenca) iz 25 držav Evrope in ZDA in Slovenije.
Na naših mednarodnih simpozijih želimo tudi vidno predstaviti raziskovalne dosežke Krkinih nagrajencev. S predavanji sodelujejo Krkini nagrajenci, mladi in novi doktorji znanosti, drugi Krkini nagrajenci pa predstavljajo svoje dosežke v obliki posterjev. Letos smo v Krko povabili priznane akademike, profesorje in strokovnjake iz devetih držav: iz Rusije, Češke, Ukrajine, Hrvaške, Italije, Latvije, Finske, Srbije in Črne gore ter Slovenije.

Na 13. mednarodnem simpoziju, ki ga bo slavnostno odprl dolgoletni mentor številnih Krkinih nagrajencev, redni član Slovenske akademije znanosti in umetnosti **akademik prof. dr. Branko Stanovnik,**

bodo sodelovali naslednji vabljeni plenarni predavatelji:

Akademik prof. dr. Jurij Petrovič Nikitin iz Rusije
Profesor Nikitin je vidno uveljavljen ruski kardiolog in terapevt. Je častni kardiolog Rusije, častni član Združenja ruskih terapevtov in častni član Univerze na Aljaski.
Profesor Nikitin je direktor Znanstveno-raziskovalnega instituta za terapijo pri sibirskem oddelku Ruske akademije medicinskih znanosti. Ustanovil in vodil je fakulteto za strokovno izpopolnjevanje zdravnikov v Novosibirsku, kjer se je šolalo že nad 30.000 ruskih zdravnikov. Njegov institut spada med najpomembnejše medicinske ustanove v Rusiji. Prednostno je proučeval široko problematiko bolezni srca in ožilja na Daljnem vzhodu in v Sibiriji. Objavil je nad 600 znanstvenih del ter več monografij in patentov. Bil je mentor pri stotih disertacijah svojih študentov. Poznan in uveljavljen je v Rusiji, pa tudi v ZDA, Kanadi, na Japonskem in drugje po svetu.

Prof. dr. Željko Reiner iz Hrvaške
Željko Reiner je redni profesor Univerze v Zagrebu in predstojnik Klinike za notranje bolezni KBC na Rebru. Je redni član Medicinske akademije Hrvaške.
Profesor Reiner je doma in v svetu cenjen vrhunski strokovnjak za kardiologijo, patofiziologijo, diabetologijo, endokrinologijo in druga področja medicine. Objavil je 270 znanstvenih člankov v priznanih mednarodnih revijah in že 10 knjig. Profesor Reiner je urednik ali član pomembnejših medicinskih revij na Hrvaškem, je tudi vabljeni profesor in zdravnik na Oddelku za interno medicino v bolnišnici Eppendorf v Hamburgu.
Profesor Reiner je danes predsednik Hrvaškega društva za aterosklerozo in član predsedstva Evropskega društva za aterosklerozo. Bil je tudi hrvaški minister za zdravstvo in član izvršnega odbora WHO. Je član številnih medicinskih društev, združenj in asociacij v svetu in doma.

Prof. dr. Jaromír Hradec iz Češke
Jaromír Hradec je izredni profesor na Prvi fakulteti za medicino v Pragi.
Je vodja Sektorja za kardiologijo na Oddelku za medicino Fakultete in glavne bolnice, Karlove univerze v Pragi.
Profesor Hradec je vidno uveljavljen češki kardiolog in predsednik češkega kardiološkega združenja in član odbora in tudi poverilnega komiteja Evropskega združenja za kardiologijo.

Njegova ožja znanstveno-raziskovalna področja so bolezni srca in ožilja, ehokardiografija, hormonsko inducirane kardiomiopatije, kardiovaskularna farmakoterapija in drugo.
Pri svojem obširnem znanstveno-raziskovalnem delu je objavil preko 250 člankov in preko 300 referatov. Aktivno sodeluje pri 15 glavnih mednarodnih kliničnih preiskavah in je nacionalni koordinator pri projektih CHARM, BETACAR, I-PRESERVE in CORONA.

Prof. dr. Pavel Poredoš iz Slovenije
Pavel Poredoš je redni profesor interne medicine na Medicinski fakulteti Univerze v Ljubljani.
Je eminenten znanstvenik in uveljavljeni strokovnjak na področju medicine, posebej na področjih perifernih arterijskih okluzivnih bolezni, mikrocirkulacije in trombolitičnega tretmaja PAOD. Raziskuje prednostno na področjih ateroskleroze, mikrocirkulacije in na detekcijah ateroskleroznih vaskularnih sprememb. Objavil je nad 200 člankov v domačih in tujih strokovnih revijah in je tudi avtor dveh knjig.
Profesor Poredoš je vodja kliničnega oddelka za vaskularne bolezni. Poleg tega je tudi predsednik Zdravniškega društva Slovenije, predsednik Centralnega evropskega vaskularnega foruma, podpredsednik delovne skupine za periferno cirkulacijo Evropskega združenja za kardiologijo in član izvršnega odbora Mednarodne zveze za angiologijo.

Prof. dr. Leonid Voronkov iz Ukrajine
Profesor Voronkov je vodja Oddelka za srčna obolenja na Institutu za kardiologijo v Kijevu. Je univerzitetni profesor za kardiologijo, predsednik Združenja kardiologov Ukrajine in član delovne skupine za srčna obolenja Evropskega združenja za kardiologijo.
Njegovo osnovno znanstveno in strokovno področje je diagnoza in zdravljenje kroničnih obolenj srca in ožilja posebno z betablokatorji. Objavil je preko 250 publikacij in je uveljavljen in priznan strokovnjak na področju interne medicine in kardiologije.

Prof dr. Ruta Muceniece iz Latvije
Ruta Muceniece je izredna profesorica na Fakulteti za medicino na Latvijski univerzi v Rigi.
Profesorica Muceniece, ki je magistrirala iz farmacije in doktorirala iz biologije, se v Latviji in tudi v svetu uveljavlja s svojimi raziskovalnimi pedagoškimi dosežki iz področij molekularne farmakologije, farmakognozije, farmacije in biosinteze.
Objavila je 132 člankov in bila avtorica 7 patentov. Profesorica Muceniece je članica pomembnih strokovnih združenj, med drugim je članica Latvijskega senatnega revizijskega komiteja, voditeljica »Trade Union Group« na Fakulteti za medicino in direktorica farmacevtskega programa.

Prof. dr. Arto Olavi Urtti iz Finske
Arto Urtti je redni profesor biofarmacije na univerzi Kuopio na Finskem. Vrsto let se je izpopolnjeval v ZDA, kjer je tudi gostujoči profesor. Je vrhunski strokovnjak na področjih sodobne biofarmacije, farmakogenomike, nanokemije, oblikovanja in transporta zdravil. Je urednik European Journal of Pharmaceutical Sciences in član odborov številnih mednarodnih znanstvenih revij iz področij farmacije in medicine. S področij oblikovanja in transporta gensko osnovanih zdravil, transdermalnega in okularnega transporta zdravil in računalniških farmakokinetičnih modelov je objavil 150 znanstvenih člankov, 14 preglednih publikacij in je avtor 19 patentov. Je mentor pri številni doktorskih in magistrskih delih svojih študentov. Na Finskem in v svetu zavzema pomembne znanstveno-ekspertne funkcije in dolžnosti. Vodi številne raziskave, ki so za Finsko ključnega pomena.

Prof. dr. Saverio Florio iz Italije
Saverio Florio je redni profesor za organsko kemijo na Fakulteti za farmacijo univerze v Bariju.
Bil je predsednik sekcije za organsko kemijo v Italijanskem kemijskem društvu in direktor meduniverzitetnega konzorcija za nove inovativne procese v sintezi. Je tudi član Znanstvenega svetovalnega odbora IASOC.
Profesor Florio se je uveljavil s svojimi raziskavami in številnimi publikacijami na področju kemije heterocikličnih spojin, organokovinskih reagentov, stereokemije in asimetrične sinteze.

Prof. dr. Darko Ivanović iz Srbije in Črne gore
Na simpoziju bo prisostvoval tudi Darko Ivanović, ki je redni profesor za analizo zdravil in dekan Fakultete za farmacijo na Univerzi v Beogradu.
Je ugleden znanstvenik in vrhunski strokovnjak na področju farmacije in analize zdravil. Objavil je preko 120 mednarodnih publikacij, sodeloval s 70 referati na mednarodnih simpozijih. Je tudi avtor 4 univerzitetnih učbenikov in knjig. Poleg izredno bogate znanstvene prakse zavzema kot vrhunski

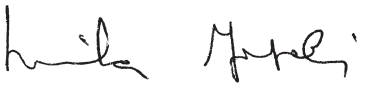
strokovnjak v svoji državi mnoge ključne položaje, je predsednik Republiškega ekspertnega komiteja za farmacijo, ustanovitelj in predsednik Sekcije za preverjanje in kontrolo farmacevtskih proizvodov pri Srbskem farmacevtskem društvu, je ustanovitelj in glavni urednik strokovno informativne revije INPHARM, bil je tudi predsednik Zvezne komisije za zdravila in tudi član strokovnih društev za farmacijo in kemijo.

Svoja doktorska dela bo predstavilo s krajšimi predavanji in v poster sekciji tudi 16 Krkinih nagrajencev, novih doktorjev znanosti.

V posebni »Poster sekciji« pa bodo ostali Krkini nagrajenci v obliki posterjev predstavili raziskovalne dosežke iz svojega dodiplomskega in magistrskega študija. Svoje raziskovalne dosežke bodo na »Poster sekciji« prikazali tudi najmlajši Krkini nagrajenci, dijaki iz dolenjskih srednjih šol.

Vsem vabljenim plenarnim predavateljem, vsem mentorjem in vsem Krkinim nagrajencem se prisrčno zahvaljujemo, ker so s svojo ustvarjalno energijo prinesli v Krko novo znanje, novo kakovost in novo ustvarjalno moč, kar je v teh spremenljivih časih tako zelo pomembno.

Predsednik znanstvenega odbora
13. mednarodnega simpozija
prof. dr. Miha Japelj



13th International Symposium and 33rd Krka Prizes

Krka Prizes are dedicated especially to motivation of young students towards extensive study, knowledge and recognition of important 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.
We started to interlink Krka Prizes with the organization of international symposiums 13 years ago. We like to invite prominent scientists, experts and researchers from all over the world. In the past 13 years, we have invited to Krka over 90 renowned and top-level university professors, academicians and experts (among them also a Nobel Prize winner) from 25 countries from Europe, USA and Slovenia as plenary lecturers.

At our international symposiums, we also present the research results of Krka Prize winners. Students with Ph.D. degree will participate as lecturers, other prize winners will present their research results in the "Poster Session". This year we have invited to Krka renowned academicians, professors and experts from nine countries: from Russia, Czech Republic, Ukraine, Croatia, Italy, Latvia, Finland, Serbia and Montenegro and Slovenia.
At 13th International Symposium, which will be officially opened by the member of the Slovenian Academy of Sciences and Arts, **Academician Prof. Dr. Branko Stanovnik,**

the following plenary lecturers will participate:

Academician Prof. Dr. Iouri Nikitin from Russia
Academician Prof. Dr. Iouri Nikitin is a recognized Russian cardiologist and therapeutist. He is an honoured cardiologist of Russia, honoured member of the Union of Russian therapeutists and honourable member of Alaska University.
Professor Nikitin is the director of Therapeutic Scientific Research Institute at Siberian Department of Russian Academy of Medical Sciences. He was a founder and leader of the Faculty for Advanced Study for Russian physicians in Novosibirsk, where already 30.000 Russian physicians have already studied. His Institute belongs among the most important medical establishments in Russia. Priorities of his studies are problems of cardiovascular diseases in the Far East and Siberia. Professor Nikitin has published more than 600 papers and several monographs and patents. He was a supervisor of more than 100 dissertations. Professor Nikitin is well known and recognized in Russia, USA, Canada, Japan and in other countries in the world.

Prof. Dr. Željko Reiner from Croatia
Željko Reiner is a full professor of internal medicine at University of Zagreb and a chairman of the Department of Internal Medicine at University Hospital Rebro. He is a member of Medical Academy of Croatia.
Professor Reiner is acknowledged and important supreme domestic and world known expert in cardiology, patophysiology, diabetology, endocrinology and in other fields of medicine. He has published 270 scientific papers in the renowned international reviews and he was an author of 10 books. He is also an editor of important medical journals in Croatia. Professor Reiner is also visiting professor, scientist and physician in USA (Oklahoma City) and Germany (Hamburg). He is a chairman of Croatian National Academy of Sciences and Arts and a member of European Atherosclerosis Society Executive Committee. Željko Reiner is also a member of several important international and domestic medical societies. He was also Minister of Health of Croatia.

Prof. Dr. Jaromír Hradec from Czech Republik
Jaromír Hradec is an associated professor at the 1st Faculty of Medicine and a head of Division of Cardiology, 3rd Department of Medicine, Faculty General Hospital, Charles University, Prague.

He is a prominent Czech cardiologist and a president of the Czech Society of Cardiology, a member of the Board of the European Society of Cardiology and a chairman of the Credentials Committee of the European Society of Cardiology.
His main areas of scientific interest are echocardiography, mitral valve prolapse, hormonally induced cardiomyopathies, heart failure, and cardiovascular pharmacotherapy. He has published over 250 publications in medical journals, and over 300 papers presented at scientific medical meetings.
Professor Hradec participated in over 15 major multicenter international clinical trials, and he is the national coordinator of the following trials: CHARM, BETACAR, I-PRESERVE, CORONA.

Prof. dr. Pavel Poredoš from Slovenia
Pavel Poredoš is a full professor of internal medicine at the Faculty of Medicine at University of Ljubljana. He is an eminent scientist and a top-level expert in the field of medicine, especially in the fields of peripheral arterial occlusive disease, microcirculation and thrombolytic treatment of PAOD. His main research interest is directed toward atherosclerosis, microcirculation and detection of early atherosclerotic vascular changes. He has published 2 books, 200 articles in national and international (SCI) journals.
Professor Poredoš is a president of Slovenian Medical Association, president of Central European Vascular Forum, a vice-president of Working Group for Peripheral Circulation at European Society of Cardiology and a member of executive board of International Union of Angiology.

Prof. Dr. Leonid G. Voronkov from Ukraine
Leonid G. Voronkov is a professor of Cardiology and a head of Heart Failure Department at Strazhesco Institute of Cardiology, Kyiv, Ukraine. He is a member of the Working Group on Heart Failure of European Society of Cardiology and a head of the Working group on Heart Failure of Ukrainian Society of Cardiology.
Professor Voronkov is a president of Kyiv Cardiologist's Society and an associate editor of Ukrainian Journal of Cardiology. His main areas of interest are the diagnosis and management of chronic heart failure (CHF) in particular, beta-blockers and peripheral mechanisms of CHF. Professor Voronkov has contributed more than 250 papers in scientific journals and he serves as a scientific reviewer for several journals on internal medicine and cardiology.

Prof. Dr. Ruta Muceniece from Latvia
Ruta Muceniece is an associated professor in Latvian University at Faculty of Medicine in Riga. She accomplished her M.Sc. studies in pharmacy and her Ph.D. studies in biology. She has become recognized in Latvia and in the world with her research and teaching achievements in the fields of molecular pharmacology, pharmacognosy, pharmacokinetics, biosynthesis, GLP and GMP. She has published 132 scientific articles and she is an author of 7 patents. Professor Muceniece is a member of important professional associations, a member of LU Senate Revision Committee, a member of Dome of Faculty of Medicine, a chief of Trade Union group of medicine and a director of Pharmacy programme.

Prof. Dr. Arto Olavi Urtti from Finland
Arto Urtti is a full professor of biopharmaceutics at the University of Kuopio in Finland.
His education and training in the field of pharmaceutical chemistry was performed in Kuopio and in University of Kansas and Wisconsin (USA). He is a visiting professor at University of California in San Francisco (USA).
Professor Urtti is a top-level expert in the field of modern biopharmacy, pharmacogenomics, nanochemistry, formulation and delivery of gene-based drugs, transdermal and ocular drug delivery, polymeric drug delivery and pharmacokinetic computer models. He has published 150 original research papers in peer-reviewed journals, 14 international review articles and book chapters, and 19 patents. He has been a supervisor of many dissertations of his students. He is an editor of DOSIS, Journal of Pharmaceutical Sciences and a member of editorial boards of important international pharmaceutical and medical journals. Professor Urtti has a variety of expert and scientific duties which are of key importance for Finland.

Prof. Dr. Saverio Florio from Italy
Saverio Florio is a full professor of organic chemistry at the Faculty of Pharmacy at University of Bari (Italy).
He was a president of the Division of Organic Chemistry of the Italian Chemical Society and a director of the Interuniversities Consortium "Metodologie e Processi Innovativi di Sintesi". Currently he is a

member of the Scientific Advisory Board of the "Ischia Advanced School of Organic Chemistry" (IASOC School). Professor Florio's research interests concern synthesis and reactivity of heterocyclic compounds, organometallic reagents, stereochemistry, chemistry and synthetic applications of heterosubstituted carbanions and asymmetric synthesis.

Prof. Dr. Darko Ivanović from Serbia and Montenegro
Darko Ivanović is a full professor for drug analysis and the dean of Faculty of Pharmacy at University of Belgrade. He is a recognized top-level expert in the field of pharmacy and analysis of drugs. He has published more than 120 international papers and 70 reports on the international symposiums. Professor Ivanović has published also 4 university textbooks. Beside important and rich scientific praxis he has also important duties and positions: he is a president of Republic Expert Committee of Pharmacy, a president and a founder of the Section for Examination and Control of Pharmaceutical Products at the Serbian Pharmaceutical Society (FDS), a founder and an editor-in-chief of INPHARM, and a member of the Serbian Pharmaceutical Society and Serbian Chemical Society.

This year, sixteen Krka Prize winners who are also new doctors of science will present their awarded theses as short lectures and in the Poster session.

Other Krka Prize winners will present their research achievements obtained in their undergraduate and master degree studies in the Poster session. Research projects of the youngest Krka Prize winners, secondary school students from the Dolenjska region will also be presented in the Poster session.

We wish to express our warmest thanks to all the invited plenary lecturers for their contributions.

Dear Krka Prize winners,
we wish to congratulate you on your research achievements and on your Krka Prizes. We hope that they would stimulate your further efforts in widening your knowledge beyond the frontiers set by the existing research and development.

Chairman of the Scientific Board
of 13th International Symposium
Prof. Dr. Miha Japelj

33rd Krka Prizes

Programme of the 13th International Symposium

Novo mesto, 23rd October 2003

9.00 – 9.10 **Chairman's Welcome Address – Miha Japelj**

9.10 – 9.20 **Opening of the Symposium – Branko Stanovnik**

1 Plenary Lectures - Part I.

Chairman: Jože Drinovec

9.20 – 9.50 Cardio-Vascular Diseases and Hyperlipidemias in Siberia Ways of Their Correction 29
Iouri Nikitin
Therapeutic Scientific Research Institute, Siberian Department of RAMS, Novosibirsk, Russia

9.50 – 10.20 Pathophysiology of Atherosclerosis and Possibilities of Prevention 30
Željko Reiner
Department of Internal Medicine, University Hospital Rebro, Zagreb, Croatia

10.20 – 10.50 Diastolic Heart Failure 32
Jaromír Hradec
3rd Department of Medicine, Charles University, Prague, Czech Republic

10.50 – 11.20 Detection of Preclinical Atherosclerosis and its Clinical Relevance 33
Pavel Poredoš
Department of Vascular Disease, University Medical Centre Ljubljana, Slovenia

11.20 – 11.50 **Coffee Break**

Plenary Lectures - Part II.

Chairman: Miha Japelj

11.50 – 12.10 Clinical Pharmacodynamics of Coryol in Chronic Heart Failure 34
Leonid G. Voronkov
Heart Failure Department, Strazhesco Institute of Cardiology, Kiev, Ukraine

12.10 – 12.40 Melanocortin Receptor Ligands as Potential Drug Candidates 35
Ruta Muceniece
Faculty of Medicine, University of Latvia, Riga, Latvia

12.40 – 13.10 Delivery of Gene Based Drugs 36
Arto Olavi Urtti
Department of Pharmaceutics, University of Kuopio, Kuopio, Finland

13.10 – 13.40 Stereoselective Oxazoline-Mediated Synthesis of Amino Acids and Epoxylactones 37
Saverio Florio
Faculty of Pharmacy, University of Bari, Bari, Italy

13.40 – 15.00 **Lunch Break**

2 Krka Prize Winners' Presentations

Chairman: Aleš Hvala

15.00 – 15.10 Intracellular Transport of Lanosterol 14α-Demethylase and NADPH Cytochrome P450 Reductase in Mammalian Cells 41
Marko Cotman
Veterinary Faculty, University of Ljubljana, Slovenia

15.10 - 15.20 Classification of *Lactobacillus gasseri* LF221 Bacteriocins Based on Their Genetic Determinants 43
Andreja Čanžek-Majhenič
Biotechnical Faculty, University of Ljubljana, Slovenia

15.20 – 15.30 The Mixed Starter Culture Efficiency in Two-stage Bioprocess for Elimination Nitrogen Compounds from Pharmaceutical Wastewater 44
Irena Čarman
Biotechnical Faculty, University of Ljubljana, Slovenia

15.30 – 15.40 Construction of Expression Cassettes for Transient Formation of Cytokines and Growth Factors in Mammalian Cells 45
Urška Čegovnik
Faculty of Medicine, University of Ljubljana, Slovenia

15.40 – 15.50 NMR Structures of Multistranded DNA and Their Interactions with Metal Ions 46
Martin Črnugelj
National Institute of Chemistry, Ljubljana, Slovenia

15.50 – 16.00 Cholesterol – Independent Regulation of Expression of the Human Lanosterol 14α-Demethylase Gene (*CYP51*) 47
Martina Fink
Faculty of Medicine, University of Ljubljana, Slovenia

16.00 – 16.10 Synthesis of Aplysinopsins, Thioaplysinopsins and Related Compounds 48
Renata Jakše
Faculty of Chemistry and Chemical Technology, University of Ljubljana, Slovenia

16.10 – 16.30 **Coffee Break**

16.30 – 16.40 Molecular Background of Fat Tissue Formation in Pig (*Sus Scrofa*) 49
Tamara Miloševič-Berlič
Biotechnical Faculty, University of Ljubljana, Slovenia

16.40 – 16.50 Unsaturated Amino Acid Derivatives as Reagents or Substrates in Heterocyclic Synthesis 50
Franc Požgan
Faculty of Chemistry and Chemical Technology, University of Ljubljana, Slovenia

16.50 – 17.00 Cytosolic Role of Mammalian Papain-like Cysteine Proteases 51
Jerica Rozman-Pungerčar
Jožef Stefan Institute, Ljubljana, Slovenia

17.00 – 17.10 Synthesis and Dynamic Behaviour of Distillation Columns Integrated Network 52
Gorazd Sobočan
Faculty of Chemistry and Chemical Technology, University of Maribor, Slovenia

17.10 – 17.20 Enrofloxacin - the Meta-Analysis of the Efficacy of Disease Treatment for Domestic Animals 53
Leon Ščuka
Veterinary Faculty, University of Ljubljana, Slovenia

17.20 – 17.30 Modeling of Longitudinal Shortening and Transversal Deformation of Skeletal Muscle 55
Boštjan Šimunič
Faculty of Electrical Engineering, University of Ljubljana, Slovenia

17.30 – 17.40 HOG Signaling Pathway in Halophilic Black Yeast *Hortaea werneckii* 56
Martina Turk
Faculty of Medicine, University of Ljubljana, Slovenia

17.40 – 17.50 Role of p41 Isoform of Invariant Chain and Cystatin C in Regulating Cysteine Proteases in Antigen-presenting Cells 57
Valentina Zavašnik Bergant
Jožef Stefan Institute, Ljubljana, Slovenia

17.50 – 18.05 **Discussion and Final Remarks**

3 Krka Prize Winners' Poster Session

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

Different Role of Intracellular Loops of Glucagon-like Peptide-1 Receptor in Signal Transduction 61
Aljoša Bavec
Faculty of Medicine, University of Ljubljana, Slovenia

Selection of the Normalization Vector is Crucial for Correct Interpretation of the cAMP-dependent Transactivation: Example of the Human *CYP51* Gene 63
Jure Ačimovič
Faculty of Medicine, University of Ljubljana, Slovenia

Good Laboratory Practice in Preclinical Evaluation of Drugs 64
Sonja Barič
Krka, d. d., Novo mesto, Slovenia

Synthesis of Synthons for Preparation of Integrin Receptor Antagonists 65
Maja Breže
Faculty of Pharmacy, University of Ljubljana, Slovenia

Influence of Different Loop Regions and Cations on Guanine Quadruplex Formation 66
Mirko Cevec
Faculty of Chemistry and Chemical Technology, University of Ljubljana, Slovenia

Synthesis and Characterization of Platinum(II) Complexes with Various Amine Ligands 67
Peter Cvelbar
Faculty of Chemistry and Chemical Technology, University of Ljubljana, Slovenia

33.
Krkine
nagrade
33rd
Krka
Prizes

1 Plenary Lectures

Cardio-Vascular Diseases and Hyperlipidemias in Siberia; Ways of Their Correction

I. Nikitin

Therapeutic Scientific Research Institute, Siberian Department of RAMS, Novosibirsk, Russia

According to international WHO programme “MONICA”, studies “Monitoring of cardio-vascular diseases tendency and their risk factors” were carried out in Novosibirsk and Moscow during the decade 1985-1995. In the Novosibirsk centre, a representative sample of randomly chosen population was examined – 11,100 patients 25-64 years of age, with 200 patients in each age- and sex-related group.

The average level of cholesterol (CS) in men was 5.6 mmol/l and in women 5.8 mmol/l. The frequency of hypercholesteremia (HCS) according to the criterion ≥5.0 mmol/l – was present in 65% of adult population, and hypertriglyceridemia (≥ 2.0 mmol/l) in 10% of adult population. Prevalence of arterial hypertension was also very high (BP ≥ 160/95 mmHg – in 31%; ≥ 140/90 mmHg – in 50%). Morbidity and mortality from myocardial infarction and stroke in Novosibirsk were one of the highest among MONICA centres.

At the same time, it is impossible to recognize primary and secondary prophylaxis of cardio-vascular diseases in Siberia, including Novosibirsk, as satisfactory. In particular, a very low percent of persons having indications for lipid-lowering drugs actually receive them. One of the reasons is the high price of modern cholesterol-lowering drugs (statins). Because of that, physicians and patients show increased interest in generic statins, including Holetar (lovastatin) and Vasilip (simvastatin) produced by “KRKA”. Both drugs were examined in marketing clinical studies that were carried out in six authoritative clinical centres in Russia.

Vasilip was studied according to a unified protocol in 167 patients with CHD and moderate HCS or combined hyperlipidemia. All patients received Vasilip in dosage regimen 20 mg/day for 6 weeks. If target levels were not reached (CS-LDL ≤ 3.0 mmol/l) the dose was doubled. With the dose of 20 mg/day, total cholesterol level lowered for 24%, on the average CS-LDL – for 34%. Target level of CS-LDL with the dose 20-40 mg of Vasilip was reached in 66% of patients. Severe adverse drug reactions that could be dangerous to health, were not registered.

Vasilip can be recommended for HCS correction in patients with CHD or in patients at high risk of its development.

Pathophysiology of Atherosclerosis and Possibilities of Prevention

Ž. Reiner
University Hospital Rebro Zagreb, Croatia

The past decade has witnessed enormous progress in our understanding of the pathophysiological nature of atherosclerosis which begins with endothelial dysfunction, the trigger for which are risk factors such as hypercholesterolemia, smoking, hypertension, hyperhomocysteinemia, impaired glucose metabolism and possibly infectious agents such as cytomegalovirus, *Helicobacter pylori* and especially *Chlamydia pneumoniae*. Particularly important is increased concentration of plasma low density lipoproteins (LDL) rich in cholesterol, and oxidant stress. They achieve impairing of endothelial function by activating proinflammatory signalling pathways such as nuclear factor kappa B (NFkB) and by reducing the bioavailability of nitric oxide (NO). Biomechanical forces on the endothelium caused by hypertension including low shear stress from disturbed blood flow, also activate the endothelium. Hypertension increases also the formation of hydrogen peroxide and free radicals such as superoxide anion and hydroxyl radicals in plasma and these substances reduce the formation of NO by the endothelium and increase leukocyte adhesion.

NFkB signal transduction pathway is an important regulator of the transcription of a number of proinflammatory genes, including those that lead to the expression of adhesion molecules, e.g. VCAM-1, ICAM-1, and selectins. High density lipoprotein (HDL) and cholesterol it contains is on the other side a protective factor for atherosclerosis, and has opposing effects on the endothelium preventing endothelial vasomotor dysfunction and reducing the expression of adhesion molecules. In response to lipoprotein oxidation, monocytes and lymphocytes are recruited to the artery wall. This involves the expression of adhesion molecules chemotactic proteins, and growth factors for monocyte – macrophages. After subsequently penetrating beneath the endothelium, monocytes transformed into macrophages accumulate oxidized lipoproteins and turn into foam cells. This process is mediated by cell surface receptors that recognize oxidatively modified LDL. Foam cells are the hallmark of early atherosclerosis. The collections of foam cells together with T lymphocytes on vessel surface could be visible to the naked eye as fatty streaks - flat yellow-gray areas.

Undamaged endothelium has an established capacity to prevent platelets aggregating into microthrombi and to regulate lipid entry into vessel walls. However, once damaged, its capacities in these respects are reduced, so aggregation of platelets occurs. Aggregation of platelets leads to release of PDGF which stimulates the migration and proliferation of smooth muscle cells (SMC). Macrophages and endothelium as well as T lymphocytes also release growth factors and cytokines (eg. PDGF, TGF–beta etc.) and are also responsible for migration of SMC from the arterial media into the intima. This migration is followed by intense proliferation of SMC with loss of their normal contractile function and an increase in synthetic function. Increased lipid uptake occurs simultaneously with an increase in SMC number, so that formation of foam cells from SMC is common too. SMC start to synthesize and secrete collagens, elastin and complex proteoglycans thus transforming a lipid lesion into a fibro-lipid atheromatous plaque. Fibroblast proliferation, which is also enhancend under these circumstances, leads also to the synthesis and secretion of collagens, and related macromolecules as well, participating therefore in the genesis of the plaque.

Fibro-lipid plaque is the characteristic lesion of advanced atherosclerosis. It consists of a cap of fibrous tissue and SMC which confers mechanical stability of the plaque and separates the lipid rich thrombogenic core from the vessel lumen and circulating blood. Influx of inflammatory cells and activation of macrophages at the plaque shoulders which then release metalloproteases and other proteolytic enzymes causes fibrous cap fissuring and thrombus formation. Non-occlusive thrombus formation in coronary arteries are usually clinically presented as unstable angina pectoris. However, deep ruptures resulting in occlusive thrombus formation in coronary arteries are the usual cause of acute myocardial infarction.

Whether a plaque will remain intact and therefore stable or rupture and lead to occlusive thrombus formation in a coronary artery causing an acute coronary syndrome due to ischemia, depends upon a number of factors, the most important of which is its composition. Stable plaques have a thick fibrous cap, a small lipid core, and few inflammatory cells. In contrast, unstable vulnerable plaques have a high lipid content, numerous inflammatory cells, and a thin fibrous cap with reduced collagen and vascular SMC in it. Although unstable plaques are believed to account for only a small number of all coronary

arteries atheromas, they are responsible for most acute coronary events.
The results of recent large clinical trials have shown that antilipemic drugs HMG-CoA reductase inhibitors (statins) do not only significantly reduce LDL cholesterol and increase HDL cholesterol but also reduce cardiovascular morbidity and mortality. However, their beneficial effects could not only be attributed to their cholesterol-lowering effects. Namely, they also reduce the enhanced susceptibility to oxidation of LDL particles, restore endothelial dysfunction, prevent arterial smooth muscle cell migration and proliferation, have antiinflammatory properties, reduce platelet aggregation, improve impaired fibrinolysis and stabilize the plaque. Therefore statins today should not be considered only as antilipemics, but as antiatherosclerotic drugs.

Diastolic Heart Failure

J. Hradec
Charles University, Prague, Czech Republic

Epidemiological studies from recent years are showing that 30-50% of all patients with chronic heart failure have a normal or almost normal left ventricular ejection fraction. It is presumed that left ventricular diastolic dysfunction is the primary cause of heart failure in these patients. Nevertheless, part of the patients have symptoms (dyspnoe, peripheral oedema) caused by extracardiac diseases (such as obstructive lung disease, obesity, venous insufficiency etc.). This makes clinical diagnosis of diastolic heart failure inaccurate.
According to the European Society of Cardiology Working Group on Heart Failure definition there are three criteria to be fulfilled for the diagnosis of diastolic heart failure: (1) signs and symptoms of heart failure; (2) preserved left ventricular systolic function (EF>0,45); (3) an objective evidence of abnormal relaxation, distensibility and/or left ventriculaar filling. But all these three diagnostic criteria are questionable. Symptoms of heart failure have very low both, specificity and positive predictive value. Systolic dysfunction can be only temporary due to e.g. decompensated hypertension, ischaemia, arrhythmias etc. Most problematic is the objective evidence of left ventricular dysfunction. There is no such simple parameter for diastolic heart function like ejection fraction is for systolic function. Practically all of the routinely used noninvasive parameters and methods (mostly derived from Doppler echocardiography) have inherited serious limitations.
Prevalence of diastolic heart failure is increasing with age. There are more females (about 60%), less post-MI patients (about 20%) and more hypertension (60-80%) among diastolic heart failure patients compared to systolic heart failure ones. Prognosis of diastolic heart failure seems to be a little better than that of systolic heart failure. Mortality is lower by about 30% in most of the epidemiological studies. Cause specific mortality also differs. There are less deaths from progression of heart failure and more non-cardiovascular deaths in diastolic heart failure. Morbidity (expressed as hospitalization rate) is about the same in both, systolic and diastolic heart failure. But again, the reasons for hospitalization are different with more hospitalizations for non-cardiovascular reasons and less ones for worsening of heart failure in diastolic heart failure.
Contrary to systolic heart failure, there are missing therapeutic mortality/morbidity clinical trials in diastolic heart failure. Therefore, treatment of this clinical entity still remains empiric. There are several ongoing therapeutic clinical trials in patients with diastolic heart failure with results of the first one (CHARM) expected this year.

Detection of Preclinical Atherosclerosis and its Clinical Relevance

P. Poredoš
University Medical Centre Ljubljana, Slovenia

Atherosclerosis is a chronic and progressive disease with relatively poorly defined ethiopathogenesis, but there are known factors (risk factors) which promote its development. In the advanced form of the disease the effect of treatment is very limited and even preventive measures do not heal the disease. The maximum potential for prevention and reversiblity of atherosclerosis would be expected with interventions at an early stage of the disease, before atherosclerotic lesions are established. Therefore, apart from identification of risk factors for atherosclerosis, the detection of early functional and structural changes of the arterial wall is of the utmost importance for prevention of progression of atherosclerosis. Newer ultrasonographic methods are of great utility for assessing the arterial wall morphology and vascular function. B-mode ultrasound provides quantitative information on morphological changes of the arterial wall and is particularly suitable for noninvasive measurement of its thickness and its consistency. For noninvasive ultrasound, investigation of the carotid arteries (CA) are the most accessible, therefore these arteries are the focus of several investigations.

There is growing information that thickening of the arterial wall intima is the earliest detectable morphological change and that it may precede the atherosclerotic lesions. Intima media thicknes (IMT) is closely related to the presence of risk factors for atherosclerosis, to their number, intensity and duration. The interrelationship between risk factors and IMT is also supported by the decrease or reduction of IMT progression with elimination of risk factors. Further it was shown that increased IMT represents the risk for the development of CV incidents.

Recent evidence also suggests that endothelial dysfunction characterized by impairment of vasodilation is the earliest detectable functional disorder in atherogenesis and may precede morphological changes of the arterial wall. Reduced vasodilation has been reported in subjects with different risk factors and in patients with atherosclerotic disease. Further it was shown that there is close interrelationship between endothelial dysfunction and cardiovascular events.

Therefore, measurement of arterial wall thickness and determination of endothelial function seems to be useful for studying the relation of risk factors to atherosclerosis and for quantifying the dose-response relation of risk factors to atherosclerosis and its progression. Finally, this techniques also represent useful tool for risk assessment.

Clinical Pharmacodynamics of Coryol in Chronic Heart Failure

L. G. Voronkov
Strazhesco Institute of Cardiology, Kyiv, Ukraine

It was shown that carvedilol, due to its unique pharmacologic properties (non-selective β-blockade, α_1-blockade, free radical scavenger effect) can remove abnormal changes at all levels of CHF - molecular, cellular, tissue/organ, and regulatory. The clinical pharmacodynamics of Coryol was studied in 24 pts wits congestive heart failure (CHF) and left ventricular ejection fraction (LVEF) < 40 % with sinus rhythm chronically treated by ACE-inhibitor and diuretic.
Coryol (open-label) was started from 3,125 mg b.i.d. and then up-titrated to target dose 25 mg b.i.d. during 8 weeks followed by maintenance phase (6 months; average 37,8 mg daily).
Long-term Coryol administration associated with significant improvement of NYHA-status ($p < 0.001$) and LVEF ($p = 0.04$), reduction of end-systolic volume ($p = 0.006$), increase of 6-minute walk test distance ($p < 0.001$). After 6 months of Coryol we observed the significant increase of heart rate variability parameters: time-domain (SDNN-i, SDANN, TI, RMSSD, pNN50) and spectral (TP, VLF, LF, LF/HF) suggested of autonomic and (possible) baroreflex function improvement. The marked decrease (approximately 3-fold) of most important anti-inflammatory cytokine - TNFα plasma level as well as significant increase of dry body mass index were also revealed.
Thus, the long-term Coryol treatment produce pronounced beneficial clinical, hemodynamical and regulatory changes in CHF, which are substantial in regard to CHF progression.

Melanocortin Receptor Ligands as Potential Drug Candidates

R. Muceniece

Faculty of Medicine, University of Latvia, Riga, Latvia

The cloning of five different subtypes of human melanocortin (MC) receptors named MC1-5 during years 1992-1994 have provided new opportunities for the drug design. Endogenous MC have long been known to elicit a large number of pleiotropic effects, for example, skin pigmentation, cardiovascular effects, the control of feeding behaviour, sexual behaviour, haemodynamics, immune regulation, nerve growth and regeneration and neuroendocrine regulations. Discovery of the two endogenous MC receptor antagonists, namely, agouti signalling peptide (ASIP) and agouti related protein (AGRP) have added new possibilities for therapeutic applications. Thus, MC receptor signalling is unique because it is mediated by the endogenous agonists and antagonists (1).

All five MC receptor subtypes are G-protein coupled receptors, leading to the stimulation of the cAMP generation after ligand activation. However, all signal transduction pathways of the MC receptors have not been completely clarified yet.

Radioligand binding studies on cells naturally expressing MC receptors and cells artificially transfected by corresponding receptor DNA or infected by baculoviruses carrying appropriate MC receptor gene, showed following affinity potency order of the endogenous peptides to MC receptors: MC1>MC3>MC4>MC5.

Nowadays a large number of MC receptor agonists and antagonists are screened for their ability to bind selectively to the MC receptor subtypes. In the past mainly linear and cyclic peptides were patented and offered for the clinical trials. Today number of a small molecular weight non-peptide compounds have appeared in the patent literature. Based on the knowledge obtained by knock-out animals and expanded pharmacological studies MC receptor ligands are promising candidates for the drug discovery. For instance, MC1 agonists could be effective for the treatment of various inflammatory conditions, MC3 agonists - for treatment of sexual dysfunctions and inflammation, MC4 receptor agonists - for treatment of obesity, antagonists – for anorexia, MC5 receptor antagonists may be useful in decreasing sebum production while agonists may be useful in increasing glandular secretion and lipolysis in adipocytes. MCs are reported to antagonize opiate dependence and tolerance possibly acting via MC4 receptor pathway. Moreover, MC mimetics chemically being small cyclic peptides and organic compounds are proved to penetrate blood-brain barrier that increases their clinical acceptance.

Discoveries in the MC field have resulted in more than 40 patents (patented by Melacure Therapeutics, Quandrant Holdings, Houghten Pharmaceutcals, Trega Biosciences, Merck & Co, etc.) started from the genes encoding MC receptors to novel compounds acting via these receptors (2,3).

Although considerable recent progress has been seen in the finding of the MC mimetics, studies on the mechanisms underlying the many actions of the MC related compounds that appear to be mediated via different distinct pathways could potentially lead to a more new opportunities in the drug design.

1. Wikberg, J.E.S., Muceniece, R., Mandrika, I., Prusis, P., Lindblom, J., Post, C., Skottner, A. New aspects on the melanocortins and their receptors. *Pharmacol Res.* **2000**, 42, 393-420.
2. WO0035942A2. 06/22/2000. Wikberg, J., Mutulis, F., Muceniece, R., Mutule, I., Schioth, H. Compounds for control of eating, growth and body weight.
3. WO09957148A1. 11/11/1999. Szardenings, M., Muceniece, R., Mutule, I., Mutulis, F., Wikberg, J. Melanocortin 1 receptor selective compounds.

Delivery of Gene-based Drugs

A. O. Urtti
University of Kuopio, Kuopio, Finland

Gene based drugs are based on nucleotide chemistry. They are either oligonucleotides or larger plasmid DNA molecules that act in the cells by suppressing the expression of the target gene (antisense, antigene, siRNA, ribozymes) or by introducing gene expression (gene therapy). Gene based drugs offer substantial possibilities in medicine, but their use is inhibited by their poor delivery into the target cells and tissues. This is due to their large molecular weight and multiple negative charges.
Cationic carriers are a non-viral strategy for gene delivery. Cationic lipids and polymers can bind and condense DNA to complexes that enhance the transgene delivery. Delivery of oligonucleotides and plasmid DNA have common problems but it seems that the same carriers are not the solution for both cases. For example, some polymeric carriers work in plasmid delivery but not for oligonucleotides.
We have investigated the mechanisms of plasmid DNA delivery into cells. Particular attention was paid to the role of glycosaminoglycans in the extracellular matrix and cell surface as barriers of gene delivery. It appears that these polysaccharides can inhibit DNA delivery by the cationic carriers. Furthermore, the role of cell cycle was investigated. Cell cycle affects the gene delivery both at the levels of cellular uptake and intracellular activity. Furthermore, once transfected the differentiated non-dividing cells can express the transgene for prolonged periods of even two months.
Overview of non-viral gene delivery systems and the mechanisms of gene delivery will be given.

1. Hyvönen, Z., et al., *Biochem. Biophys. Acta* **2000,** 1509, 451-466.
2. Hyvönen, Z., et al., *Eur. J. Pharm. Sci.* **2002,**15, 449-460.
3. Jääskeläinen et al., *Eur. J. Pharm. Sci.* **2000,** 10, 187-193.
4. Ruponen, M., et al., *Biochem. Biophys. Acta* **1999,** 1415, 331-341.
5. Ruponen, M., et al., *J. Biol. Chem.* **2001,** 276, 33875-33880.

Stereoselective Oxazoline-Mediated Synthesis of Amino Acids and Epoxylactones

S. Florio
Faculty of Pharmacy, University of Bari, Bari, Italy

Since its descovery going back to more than 100 years ago, the oxazoline system never stopped offering the organic chemists synthetic opportunities for the preparation of a variety of substances of biological importance, particularly over the past 35 years.[1]
Among the various aspects of the chemistry of the oxazoline system that dealing with the generation and synthetic exploitation of metallated 2-alkyloxazolines has been extensively pursued. Instead, the chemistry of metallated heterosubstituted 2-alkyloxazolines has been much less studied.[2] Few papers concerned with the generation of lithiated 2-chloroalkyloxazolines and some synthetic applications have recently come from our research group in Bari.[3]
This lecture will be mainly focused on stereoselective syntheses using methodologies based on simple oxazoline bearing reactive intermediates. It will be shown how easily available lithiated heterosubstituted 2-alkyloxazolines add cleanly and smoothly to nitrones to generate highly strained intermediates which are further elaborated to amino acids and to carbonyl compounds to produce epoxylactones which are useful building blocks for the preparation of biologically important target molecules. Special emphasis will be given to the oxiranyl anions based methodology.

1. Meyers, A.I. *J. Heterocyclic Chemistry*, **1998**, 35, 991.
2. Fustero, S., Salavert, E., Navarro, A., Mojarrad, F., Fuentes, A.S. *Targets in Heterocyclic Systems* **2001**, 5, 235.
3. Capriati, V., Degennaro, L., Florio, S., Luisi, R., Tralli, C., Troisi, L. *Synthesis*, **2001**, 15, 2299. and Florio, S., Capriati, V., Luisi, R., Abbotto, A., Pippel, D.J. *Tetrahedron* **2001**, 57, 6775 and Refs. Therein.

33. Krkine nagrade
33rd Krka Prizes

2 Krka Prize Winners´ Presentations

33

Intracelularni transport lanosterol 14α-demetilaze in NADPH-citokrom P450 reduktaze v celicah sesalcev

M. Cotman
Veterinarska fakulteta, Univerza v Ljubljani, Slovenija
Mentorica: D. Rozman
Medicinska fakulteta, Univerza v Ljubljani, Slovenija
Somentor: G. Majdič
Veterinarska fakulteta, Univerza v Ljubljani, Slovenija

Lanosterol 14α-demetilaza (CYP51) je encim, ki spada v naddružino citokromov P450 in je vključen v postskvalenski del biosinteze holesterola. CYP51 odstrani iz lanosterola 14α-metilno skupino, pri čemer nastane FF-MAS (angl. folicular fluid meiosis activating sterol). FF-MAS lahko ponovno sproži mejozo v mišjih oocitih v pogojih *in vitro*, kar nakazuje možnost njune vlogo med oploditvijo. Protein CYP51 pri sesalcih je mikrosomalni citokrom P450, ki se izraža v vseh tipih celic. Protein CYP51 v mišjih jetrih se zadržuje predvsem na membranah gladkega endoplazemskega retikuluma (gER). Značilno velike količine proteina CYP51 smo določili tudi na Golgijevem aparatu (GA), nismo pa ga našli na celični membrani. V mišjih haploidnih moških celicah protein CYP51 ni prisoten samo na Golgijevem aparatu in na ER, ampak tudi na zunanji in notranji membrani akrosoma. Protein CYP51 smo zasledili v vseh fazah razvoja akrosoma. Določen je bil tudi v akrosomalnih membranah iz ejakuliranega semena bikov in ovnov, kar kaže razvojno ohranjeni mehanizem stabilizacije proteina. Protein CYP51 velikosti 53 kDa prevladuje predvsem v jetrnih mikrosomih miši, na *cis*-, *medialni* in *trans*-Golgijevi frakciji pa se pojavi oblika velikosti 70 kDa. Pri miši z enodnevnim postom oblika 70 kDa izgine, vendar še vedno zasledimo protein velikosti 53 kDa. Na akrosomalnih membranah, izoliranih iz ovnovega in bikovega semena, smo določili imunoreaktivne proteine CYP51 velike 50 kDa, 60 kDa, 70 kDa, 90 kDa in 120 kDa, ki se pojavljajo z različno intenziteto. Imunoreaktivni protein CYP51 z velik 70 kDa ni ubikvitiniran, je pa glikoziliran. Ti podatki kažejo, da lahko na promet citokromov P450 vplivajo celično specifični mehanizmi in posttranslacijske modifikacije. CYP51 je prvi citokrom P450 protein, ki se nahaja na akrosomu.

Intracellular Transport of Lanosterol 14α-demethylase and NADPH Cytochrome P450 Reductase in Mammalian Cells

M. Cotman
Veterinary Faculty, University of Ljubljana, Slovenia
Supervisor: D. Rozman
Faculty of Medicine, University of Ljubljana, Slovenia
Co-supervisor: G. Majdič
Veterinary Faculty, University of Ljubljana, Slovenia

Lanosterol 14α-demethylase (CYP51) is a cytochrome P450 enzyme involved in the posqualene portion of cholesterol biosynthesis. CYP51 removes 14α-methyl group from lanosterol forming FF-MAS (folicular fluid meiosis activating sterol). FF-MAS stimulates the reinitiation of meiosis in mouse oocyte *in vitro* and is belived to have an important role in fertilisation. Mammalian CYP51 is a ubiquitously expressed microsomal cytochrome P450. In the mouse liver, CYP51 resides primarily on membranes of the smooth endoplasmic reticulum (SER). Significant amount of the protein is detected also in Golgi apparatus (GA), but not on plasma membrane. In mouse haploid male germ cells, CYP51 resides not only on SER and GA but also on the outer and inner membranes of acrosome. CYP51 is detected during all phases of acrosome development. It is also detected on acrosomal membrane from bull and ram ejaculated sperm, indicating evolutionarily conserved mechanism for its longterm storage/stabilization. The expected 53 kDa immunoreactive CYP51 predominates in the liver microsomes. However, on *cis*- , *medial*- and *trans*- Golgi a 70 kDa form is also found. In mouse liver after 24-hour starvation the 70 kDa form disappeared, while the 53 kDa form persisted. Acrosomal membranes isolated from ram and bull sperm contain 50 kDa, 60 kDa, 70 kDa, 90 kDa and 120 kDa CYP51-immunoreactive proteins that are detected

with different intensity in different preparations. The 70-kDa CYP51 immunoreactive protein appears not to be ubiquinated but seems to be glycosilated. These data show for the first time that cell type-specific mechanisms and post-translational modifications may influence trafficking of cytochrome P450 proteins. CYP51 is the first cytochrome P450 to be detected on the acrosomal membrane.

Klasifikacija bakteriocinov seva *Lactobacillus gasseri* LF221 na osnovi genskega zapisa

A. Čanžek Majhenič
Biotehniška fakulteta, Univerza v Ljubljani, Slovenija
Mentorica: I. Rogelj
Somentorica: D. Žgur Bertok
Biotehniška fakulteta, Univerza v Ljubljani, Slovenija

Lactobacillus gasseri LF221, predhodno identificiran kot *Lactobacillus acidophilus* LF221, proizvaja vsaj dva bakteriocina, imenovana acidocin LF221 A in acidocin LF221 B. Ker gre za zelo zanimiv, potencialno probiotičen sev, smo želeli natančno raziskati tudi njegove bakteriocine, in sicer na nivoju genskega zapisa. Genske determinante za acidocina LF221 A in B smo ugotovili na *Bam*HI/*Hind*III, oziroma *Eco*RI/*Hind*III odsekih kromosomske DNA seva LF221 in jih uporabili v reakcijah ugotavljanja nukleotidnega zaporedja. Analize rezultatov nukleotidnega zapisa so potrdile domneve predhodnih biokemijskih raziskav in delnega aminokislinskega zaporedja očiščenih peptidov, da sta acidocina LF221 A in B predstavnika II. skupine bakteriocinov. V primeru acidocina LF221 A gre za nov in še ne opisan bakteriocin, medtem ko je acidocin LF221 B zelo podoben gasericinu T. Podrobnejše analize so razkrile, da gre za dva dvopeptidna bakteriocina. Poleg tega smo z iskanjem podobnosti zaporedij acidocinov LF221 A in B z že opisanimi bakteriocini našli delne sorodnosti le z dvopeptidnimi bakteriocini. Rezultati dobljenih sorodnosti tudi namigujejo, da je acidocin LF221 A morda odgovoren za pretežni del inhibitornega delovanja seva LF221, kar pa bomo lahko potrdili le z ekspresijo posameznega acidocina LF221. Zaradi širokega protimikrobnega spektra, tvorbe toplotno stabilnih, majhnih, dvopeptidnih bakteriocinov ter probiotičnih lastnosti je sev LF221 zelo obetaven mikrob z možnostjo uporabe v proizvodnji fermentirane hrane in farmacevtskih preparatih.

Classification of *Lactobacillus gasseri* LF221 Bacteriocins Based on their Genetic Determinants

A. Čanžek Majhenič
Biotechnical Faculty, University of Ljubljana, Slovenia
Supervisor: I. Rogelj
Co-supervisor: D. Žgur–Bertok
Biotechnical Faculty, University of Ljubljana, Slovenia

Lactobacillus gasseri LF221, previously identified as *Lactobacillus acidophilus* LF221, produces at least two bacteriocins, namely acidocin LF221 A and acidocin LF221 B. LF221 was already proven to be an interesting microbe with potential probiotic properties. To get more useful information about LF221 bacteriocins on the genetic level, molecular biology tools were used to locate genetic determinants coding for acidocin LF221 A an B, respectively. *Bam*HI/*Hind*III and *Eco*RI/*Hind*III fragments, carrying genes for LF221 acidocins, respectively, were subjected to the sequencing reactions. Analyses of the nucleotide sequences confirmed LF221 acidocins as members of II. class bacteriocins, where acidocin LF221 A was proven to be new bacteriocin, and acidocin LF221 B virtually identical to gassericin T. Biochemical characteristics and partial amino acid sequences of purified peptides predicted for same classification. Moreover, the nucleotide sequence revealed that acidocins LF221 A and B are two-peptide bacteriocins. Homology searches between acidocin LF221 A and B, and already described bacteriocins, confirmed them as two-peptides as both exerted homologies only to two-peptide bacteriocins. According to the results of homology searches, we predict acidocin LF221 A as a major carrier of inhibitory activity of LF221 bacteriocin complex. But only expression studies could clarify this doubt. Nevertheless, wide inhibitory spectrum, production of heat-stable, small, two-peptide bacteriocins and probiotic properties define LF221 strain as a promissing microbe with possible application in food production and pharmaceutical purposes.

Učinkovitost združene starterske kulture v dvostopenjskem bioprocesu odstranitve dušikovih spojin iz odpadne vode farmacevtske industrije

I. Čarman
Krka, d. d., Novo mesto, Slovenija
Mentor: P. Raspor
Biotehniška fakulteta, Univerza v Ljubljani, Slovenija
Somentorica: M. Glancer–Šoljan
Prehrambeno-biotehnološki fakultet, Zagreb, Hrvaška

V delu obravnavamo razvoj tehnologije čiščenja, ki bo na obstoječi čistilni napravi omogočila učinkovito odstranitev dušikovih spojin iz odpadne vode farmacevtske industrije. Aktivnost združene starterske kulture (ZSK), sestavljene iz treh nitrifikacijskih (*Nitrosomonas europaea*, *Nitrosococcus mobilis* in *Nitrosovibrio tenuis*) in treh denitrifikacijskih bakterijskih vrst (*Moraxella osloensis*, *Corynebacterium mycetoides*, *Brevundimonas diminuta*) smo preizkusili v procesih odstranitve dušikovih spojin. S ciklično izvedenimi nitrifikacijami in denitrifikacijami je ZSK povečevala svojo aktivnost v procesih odstranitve dušikovih spojin, s tvorbo agregatov pa je večala tudi mikrobiološko in fiziološko stabilnost. Kemično definirano gojišče smo postopno nadomeščali s farmacevtsko odpadno vodo. S prekinjenimi in neprekinjenimi enostopenjskimi poskusi smo ugotovili, da ZSK koncentracije do 100 mg NH_4-N/l oksidira v 20 – 24 urah do vrednosti 3 – 12 mg/l. Nadaljnje delo je potekalo v smeri postavitve dvostopenjskega laboratorijskega bioreaktorskega sistema, z ločenima stopnjama za avtotrofno nitrifikacijo in heterotrofno denitrifikacijo. ZSK je v dvostopenjskem bioprocesu, pri definiranih tehnoloških parametrih dosegla zadovoljivo kvaliteto čiščene odpadne vode farmacevtske industrije in pri tem ohranjala svojo stabilno strukturo v obliki agregatov. S postavitvijo preddenitrifikacijske stopnje, ki je delovala kot selektor, se je preprečevala filamentozna razrast.

The Mixed Starter Culture Efficiency in Two – Stage Bioprocess for Elimination Nitrogen Compounds from Pharmaceutical Wastewater

I. Čarman
Krka, d. d., Novo mesto, Slovenija
Supervisor: P. Raspor
Biotechnical Faculty, University of Ljubljana, Slovenia
Co-advisor: M. Glancer–Šoljan
The Faculty of Food Technology and Biotechnology, Zagreb, Croatia

The Paper deals with the development of treatment technology (for nitrogen removal) that will enable effective nitrogen compounds removal from pharmaceutical waste water in the existing industrial wastewater treatment plant. The activity of the starter mixed culture (SMC) consisted of nitrification species of bacteria (*Nitrosomonas europaea*, *Nitrosococcus mobilis*, *Nitrosovibrio tenuis*) and denitrification species of bacteria (*Moraxella osloensis*, *Corynebacterium mycetoides*, *Brevundimonas diminuta*) were tested in process for nitrogen removal. With cyclically executed nitrification and denitrification SMC was increasing nitrogen removal activity and also microbiological and physiological stability caused by cells aggregation. Chemical defined medium was gradually substituted with pharmaceutical wastewater. In batch and continually single-stage experiments the ammonium concentrations about 100 mg/l were oxidized by SMC from 3 to 12 mg/l in 20 – 24 hours. The further work was executed in two - stage laboratory plant, where optimal conditions for nitrification and denitrification were defined in a separate reactors. In a two - stage bioprocess at defined technological parameters a SMC achieved satisfactory waste water effluent quality from pharmaceutical industry The filamentous growth was prevented with predenitrification step act as a selector.

Priprava ekspresijskih kaset za prehodno tvorbo citokinov in rastnih faktorjev v sesalskih celicah

U. Čegovnik
Medicinska fakulteta, Univerza v Ljubljani, Slovenija
Mentor: S. Novaković
Medicinska fakulteta, Univerza v Ljubljani, Slovenija

Najnovejši in najobetavnejši pristop biološkega zdravljenja raka temelji na pripravi gensko spremenjenih tumorskih celic. Za njihovo pripravo smo uporabili gene za citokine in rastne faktorje, kot so hIL2, hIFNgγ, hTNFaα, hGMCSF in hGCSF. Prva stopnja v pripravi gensko spremenjenih tumorskih celic je bila priprava ekspresijskih kaset, ki je temeljila na vnosu izbranih genov za citokine in rastne faktorje v ustrezne ekspresijske vektorje. Gene za citokine in rastne faktorje smo namnožili z metodo PCR. Ker gen hGCSF ni vseboval vodilnega zaporedja, potrebnega za izvencelično izločanje proteina, smo mu to dodali z ligacijo treh parov komplementarnih oligonukleotidov. Namnožene gene smo vnesli v ekspresijske vektorje, pripravljene ekspresijske kasete pa analizirali z metodo PCR ter z restrikcijsko in sekvenčno analizo. Ekspresijske kasete smo nadalje vnesli v mišje melanomske celice B16F1. Za vnos smo uporabili dva nevirusna vnosna sistema (elektrotransfekcijo in lipofekcijo). Po transfekciji celic smo z metodo ELISA in z biološkim testom določili prisotnost citokinov in rastnih faktorjev v gojišču celic. Najvišjo količino proteina smo ugotovili prva dva dneva po transfekciji, nato pa je njegova količina močno padla. Z biološkim testom smo dokazali, da omogočajo pripravljene ekspresijske kasete le nastanek proteina s pravilno sekundarno in terciarno strukturo.

Construction of Expression Cassettes for Transient Formation of Cytokines and Growth Factors in Mammalian Cells

U. Čegovnik
Faculty of Medicine, University of Ljubljana, Slovenia
Supervisor: S. Novaković
Faculty of Medicine, University of Ljubljana, Slovenia

One of the most recent and promising immunotherapeutic strategies for the treatment of cancer is based on the creation of genetically manipulated tumor cells. In attempts to create it, we used genes for cytokines and growth factors such as hIL2, hIFNγ, hTNFα, hGMCSF and hGCSF. The first step in the creation of genetically modified tumor cells was the construction of expression casettes. We inserted selected genes for cytokines/growth factors into suitable expression vectors. The genes for cytokines and growth factors were amplified by PCR. Since the leader sequence of the hGCSF gene was lacking (necessary for extracellular protein excretion), it was added to the gene. The leader sequence was constructed by a complementary ligation of three oligos pairs. The amplified genes were inserted into expression vectors; the constructed expression cassettes were then analyzed by PCR, restriction and sequence analysis. The expression cassettes were then transferred into the mouse melanoma B16F1 cells. We used two types of non-viral transfer system for a transfection of melanoma cells (electrotransfection and lipofection). After the cell transfection, the presence of excreted cytokines and growth factors in the cell growth medium was tested. The protein detection was measured by ELISA system and by a bioassay. The highest amount of protein was detected in the first two days after the cell transfection. The third day after transfection the amount of the protein decreased rapidly. The bioassay was performed to prove a proper secondary and terciary structure of the protein that encoded constructed expression cassettes.

NMR strukture DNK z več vijačnicami in njihove interakcije s kovinskimi ioni

M. Črnugelj
Kemijski inštitut, Ljubljana, Slovenija
Mentor: J. Plavec
Kemijski inštitut, Ljubljana, Slovenija

Zanimanje za G-kvadrupleksne DNK strukture se je v zadnjem času zelo povečalo, saj so se pojavili dokazi o vpletenosti teh struktur pri različnih pomembnih bioloških procesih.
S pomočjo NMR spektroskopije in molekulsko dinamskih izračunov smo ugotovili, da $d(G_4T_4G_3)_2$ oligonukleotidno zaporedje v prisotnosti Na^+ ionov tvori dimerno G-kvadrupleksno strukturo, sestavljeno iz treh G-kvartetov, ki so naloženi eden na drugega, in iz dveh timidinskih zank, ki potekata po diagonalah zunanjih G-kvartetov, ter dveh dodatnih gvaninov, ki se oba nahajata na isti strani G-kvadrupleksnega jedra. Ugotovili smo, da pride pri strukturi $d(G_4T_4G_3)_2$ do zdrsa fragmenta G9-G11 za eno bazo proti 3'-koncu glede na sorodno strukturo $d(G_4T_4G_4)_2$. Ta strukturni detajl poprej še ni bil eksperimentalno opažen.
Pri študiju oligonukleotidnega zaporedja $d(G_3T_4G_4)$ smo ugotovili, da tvori bimolekularni G-kvadrupleks, sestavljen iz treh G-kvartetov, dveh timidinskih zank, G3 nukleotida, ki je del zanke, in G11 nukleotida tako v prisotnosti K^+, Na^+ kot NH_4^+ ionov. G-kvadrupleks $d(G_3T_4G_4)_2$ je prvi bimolekularni G-kvadrupleks, ki vsebuje tako diagonalno zanko kot zanko, ki poteka po robu G-kvadrupleksnega jedra in predstavlja nov razred bimolekularnih G-kvadrupleksov, ki vsebujejo tri paralelne in eno antiparalelno gvanozinsko verigo ter hkrati diagonalno ter robno zanko.

NMR Structures of Multistranded DNA and their Interactions with Metal Ions

M. Črnugelj
National Institute of Chemistry, Ljubljana, Slovenia
Supervisor: J. Plavec
National Institute of Chemistry, Ljubljana, Slovenia

G-quadruplex DNA structures are presently a subject of great interest since their formation has been suggested to play a role in many important biological proceses.
Using a combination of NMR spectroscopy and restrained molecular dynamics calculations we have shown that $d(G_4T_4G_3)$ sequence forms an asymmetric dimeric G-quadruplex structure in the presence of Na^+ ions consisting of three stacked G-quartets, two T_4 loops that span diagonally across the outer faces of the G-quartets and two guanine residues G4 and G12, which reside on the same side of the G-quadruplex core. The structure of $d(G_4T_4G_3)_2$ exhibits a "slipped-loop" element that has not been experimentally observed before.
Our study has shown that $d(G_3T_4G_4)$ oligonucleotide sequence forms an asymmetric G-quadruplex structure composed of three G-quartets, two tymidine loops, G3 residue which is a part of the loop and overhanging G11 residue in the presence of K^+, Na^+ as well as NH_4^+ ions. This is the first bimolecular G-quadruplex comprised of diagonal as well as edge type loops. $d(G_3T_4G_4)_2$ G-quadruplex represents a new category of bimolecular G-quadruplexes having three parallel and one antiparallel strand and diagonal as well as edge type loops.

Od holesterola neodvisno uravnavanje izražanja človeškega gena za lanosterolno 14α-demetilazo (*CYP51*)

M. Fink
Medicinska fakulteta, Univerza v Ljubljani, Slovenija
Mentorica: D. Rozman
Medicinska fakulteta, Univerza v Ljubljani, Slovenija

Lanosterol-14α-demetilaza (*CYP51*) sodeluje v ''hišni poti'' biosinteze holesterola pri živalih. Promotorska področja sesalskih genov *CYP51* so visoko evolucijsko ohranjena in vsebujejo številna regulatorna zaporedja DNA, kot so od cAMP odvisni regulatorni elementi (CRE), s steroli uravnavani element (SRE) in element, ki veže družino transkripcijskih faktorjev Sp (GC-blok). Namen našega dela je bil pripomoči k boljšemu razumevanju molekularnih mehanizmov uravanja izražanja lanosterol-14α-demetilaze in raziskati od holesterola neodvisno uravnavanje izražanja človeškega gena *CYP51*.
Ugotovili smo, da so protein-protein interakcije med od cAMP-odvisnimi in transkripcijskimi faktorji in SREBP nujne za s SREBP-posredovano prepisovanje človeškega gena *CYP51*. Regulatorna elementa CRE2 in SRE1 posredujeta osnovno, od cAMP-odvisno in od SREBP odvisno prepisovanje, Prepisovanje gena *CYP51* se lahko z od cAMP odvisnimi agensi aktivira tudi v razmerah, ko imajo celice dovolj sterolov. Zaključimo lahko, da je uravnavanje izražanja človeškega gena *CYP51* kompleksen proces, kjer se v različnih fizioloških okoliščinah različni transkripcijski faktorji ob sodelovanju koaktivatorjev vežejo na promotor človeškega gena *CYP51* in medsebojno interagirajo preko interakcij protein-protein.

Cholesterol – Independent Regulation of Expression of the Human Lanosterol 14α-demethylase Gene (*CYP51*)

M. Fink
Faculty of Medicine, University of Ljubljana, Slovenia
Supervisor: D. Rozman
Faculty of Medicine, University of Ljubljana, Slovenia

Lanosterol 14α-demethylase (*CYP51*) is involved in the housekeeping process of cholesterol biosynthesis in animals. The promoter/regulatory regions of mammalian *CYP51* genes are highly evolutionary conserved and contain several regulatory DNA sequences: cAMP responsive elements (CRE), sterol regulatory element (SRE) and GC-box. The goal of our study was to contribute to a better understanding of molecular mechanisms involved in regulation of lanosterol 14α-demethylase (*CYP51*) gene expression. We have established that protein-protein interactions between the cAMP-dependent and sterol-dependent transcription factors are essential for *CYP51* expression. Experiments with deletion *CAT* constructs indicated that -334/-121 region is necessary for basal, cAMP-dependent and for sterol-dependent *CYP51* expression. Interestingly, a functional CRE2 is essential for sterol-dependent transcription of h*CYP51*. Transcription of the human *CYP51* gene can be activated also in sterol-repressed conditions with cAMP-dependent agents such as forskolin, or overexpression of CREMτ or CREB transcription factors. Results demonstrate that regulation of *CYP51* gene expression is a complex process where under different physiological conditions various transcription factors bind to the *CYP51* promoter and interact with each other.

Sinteze aplisinopsinov, tioaplisinopsinov in sorodnih spojin

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

Tioaplisinopsinove in aplisinopsinove derivate smo sintetizirali po treh metodah. Prva reakcijska pot je bila dvostopenjska; v prvi stopnji smo pripravili dimetilaminometilidenske derivate različno substituiranih tiohidantoinov, ki smo jih v drugi stopnji z izmenjavo dimetilaminske skupine z 2-metilindolom pretvorili v tiooksoaplisinopsinove derivate. V drugi, trostopenjski sintezi, smo 2-substituiran 3-dimetilaminopropenoat pretvorili v dva 3-(indol-3-il)propenoata. Nastalima produktoma smo odstranili *N*-zaščitno skupino. Zadnja stopnja je bila reakcija aminopropenoatov z različnimi izotiocianati in izocianati. Tretja pot preko indolkarbaldehida je vodila tako do tioaplisinopsinovih derivatov z elektronprivlačno skupino na mestu 2 v indolovem obroču kot tudi do tiohidantoin karbolinov. Z reakcijo alkil 3-indolacetatov s *terc*-butoksi-bis(dimetilamino)metanom (Bredereckovim reagentom) smo pripravili alkil 3-dimetilamino-2-(indol-3-il)propenoate. S substitucijo dimetilaminske skupine z nekaterimi *N*- in *C*-nukleofilnimi spojinami so nastali derivati α,β-nenasičenih aminokislin. Z aktiviranimi amini, ki imajo aminsko ali hidroksilno skupino na α-mestu glede na obročni dušikov ali nukleofilni ogljikov atom, poteče intramolekularna kondenzacija obročnega *N*- ali *C*-atoma na estrsko skupino propenoata, pri čemer nastanejo kondenzirani pirimidonski derivati z indolom kot prosto stransko skupino.

Synthesis of Aplysinopsins, Thioaplysinopsins and Related Compounds

R. Jakše
Faculty of Chemistry and Chemical Technology, University of Ljubljana, Slovenia
Supervisor: B. Stanovnik
Faculty of Chemistry and Chemical Technology, University of Ljubljana, Slovenia

Thioaplysinopsin derivatives were prepared following three different synthetic routes. The first route included the preparation of (dimethylamino)methylidene substituted thiohydantoin derivatives which were transformed to thioaplysinopsin analogs by substitution of dimethylamino group with 2-methylindole in acidic media. The second included two 3-(indol-3-yl)propenoates as starting compounds which reacted with indoles to give the appropriate substitution products. The latter were deprotected with Pd/C/hexene to give methyl 2-amino-3-(indol-3-yl)propenoate and methyl 2-amino-3-(2-methylindol-3-yl)propenoate. These compounds were then transformed with various alkyl- and aryl isothiocyanates into a series of thioaplysinopsin derivatives with mostly good stereoselectivity. Aplysinopsin and thioaplysinopsin derivatives with an electron-withdrawing group at position 2 in the indole moiety as well as thiohydantoin carboline derivatives were prepared via indolecarbaldehide. Ethyl amd methyl 3-dimethylamino-2-(indol-3-yl)propenoates were prepared from the appropriate indoleacetic acid esters, using *tert*-butoxy-bis(dime thylamino)methane (Bredereck's reagent). By substitution of the dimethylamino group with various *C*- and *N*-nucleophilic compounds α,β-unsaturated amino acid derivatives were formed in moderate to good yields. Intramolecular cyclisation to the ester group took place due to the annular *N*- or nucleophilic *C*-atom and this way only condensed pyrimidone derivatives were isolated.

Molekulski mehanizmi uravnavanja tvorbe maščobnega tkiva pri prašiču (*Sus scrofa*)

T. Miloševič Berlič
Biotehniška fakulteta, Univerza v Ljubljani, Slovenija
Mentor: P. Dovč
Biotehniška fakulteta, Univerza v Ljubljani, Slovenija

Izolirali smo kandidatne gene *PGC-1*, *TFAM* in *UCP-1* prašiča, ki so vključeni v uravnavanje metabolizma maščobnega tkiva, in jih karakterizirali. Z RT-PCR in z RACE smo pomnožili celotno kodirajočo regijo *PGC-1* in *TFAM* ter pripadajoči 5'- in 3'-neprevedeni regiji obeh genov. S sekvenciranjem osmega eksona *PGC-1* pri osmih različnih pasmah prašičev smo ugotovili tri genetske variante, ki smo jih lahko identificirali z analizo SSCP. S fluorescentno hibridizacijo *in situ* smo ugotovili kromosomsko lokalizacijo gena *PGC-1* na osmem kromosomu prašiča (SSC8, p2.1-2.3).
S translacijo *in vitro* v pšeničnem ekstraktu smo pripravili rekombinantni protein TFAM in ga uporabili v testu EMSA ter za sledenje z DNazo I. S sekvenciranjem potencialnih veznih mest za TFAM v D-loop regiji mtDNA različnih pasem prašičev smo ugotovili, da je ta del mtDNA polimorfen. Genotipizacija fragmentov gena *UCP-1* v gelih MetaPhor je potrdila prisotnost dolžinskega polimorfizma v 3'-neprevedeni regiji tega gena. Ugotovili smo tudi nukleotidno zaporedje celotnega introna 3 in delno nukleotidno zaporedje eksonov 3 in 4 gena *UCP-1* prašiča. Naši rezultati pomenijo prvo karakterizacijo genov *PGC-1* in *TFAM* pri prašiču in odpirajo možnost za nadaljnje proučevanje vloge teh regulatornih proteinov v procesih adaptivne termogeneze in adipogeneze.

Molecular Background of Fat Tissue Formation in Pig (*Sus scrofa*)

T. Miloševič Berlič
Biotechnical Faculty, University of Ljubljana, Slovenia
Supervisor: P. Dovč
Biotechnical Faculty, University of Ljubljana, Slovenia

Three candidate genes, porcine *PGC-1*, *TFAM* and *UCP-1* gene, which are involved in regulation of fat tissue metabolism, were isolated and characterized. Using RT-PCR and RACE techniques the entire coding region of porcine *PGC-1* and *TFAM* gene including their 5'- and 3'-UTR sequences were obtained. Sequence analysis of exon 8 of *PGC-1* in eight different pig breeds revealed three single nucleotide polymorphisms, which were confirmed by SSCP analysis as well. Using fluorescent hybridisation *in situ* we have mapped *PGC-1* gene on porcine chromosome eight (SSC8, p2.1-2.3). Recombinant TFAM was prepared in the wheat germ extract *in vitro* translation system and used in EMSA and in DNase I Footprint analyses. Sequence analysis of potential TFAM binding sites in the D-loop region of mtDNA in different pig breeds has revealed polymorphisms in this region.
By genotyping *UCP-1* fragments in Metaphor agarose the length polymorphism in the 3'-UTR of the porcine *UCP-1* gene were confirmed. The entire nucleotide sequence of intron 3 and partial nucleotide sequence of exons 3 and 4 of porcine *UCP-1* gene were determined. Our results represent first molecular characterization of porcine *PGC-1* and *TFAM* and open the possibility for further study of the role of these regulatory proteins in adaptive thermogenesis and adipogenesis.

Nenasičeni aminokislinski derivati kot reagenti ali substrati v heterociklični sintezi

F. Požgan
Fakulteta za kemijo in kemijsko tehnologijo, Univerza v Ljubljani, Slovenija
Mentor: M. Kočevar
Fakulteta za kemijo in kemijsko tehnologijo, Univerza v Ljubljani, Slovenija

Raziskoval sem različne aminokislinske derivate kot reagente ali substrate v sintezi heterocikličnih spojin. Ugotovil sem, da lahko pod določenimi reakcijskimi pogoji 4-acilhidrazinometilenoksazol-5(4*H*)-one uporabimo kot reagente za aciliranje *N*-nukleofilov. Med drugim sem tako pripravil fungicid salicilanilid. Raziskal sem regioselektivnost Schmidtove reakcije na pripojenem cikloalkanonskem obroču piran-2-onskih derivatov in ugotovil, da na razmerje produktov najbolj vpliva temperatura reakcijske zmesi. Z omenjeno reakcijo sem uspel pripraviti tudi nov heterociklični sistem, pirano[3,2-*b*]azepin. Nadalje sem raziskal pretvorbe piran-2-onskih derivatov z aminsko skupino na mestu 3 s hidrazin hidratom v 1,4-dihidropiridazinske derivate. Ugotovil sem, da različne izhodne spojine zahtevajo različne pogoje za uspešno tvorbo 1,4-dihidropiridazinskih derivatov s karbohidrazidno skupino na mestu 3. Z izolacijo nekaterih intermediatov sem predpostavil reakcijski potek pretvorb. Karbohidrazidne produkte sem z anorganskimi oksidanti (talijev(III) nitrat trihidrat, bakrov(II) acetat monohidrat, cerijev(IV) amonijev nitrat) nadalje pretvoril v estre, pri čemer je v odvisnosti od vrste in količine uporabljenega oksidanta, prišlo tudi do oksidacije 1,4-dihidropiridazinskega obroča.

Unsaturated Amino Acid Derivatives as Reagents or Substrates in Heterocyclic Synthesis

F. Požgan
Faculty of Chemistry and Chemical Technology, University of Ljubljana, Slovenia
Supervisor: M. Kočevar
Faculty of Chemistry and Chemical Technology, University of Ljubljana, Slovenia

The utility of various amino acid derivatives as reagents or substrates in heterocyclic synthesis has been investigated. It was shown that depending on reaction conditions the 4-acylhydrazinomethyleneoxazol-5(4*H*)-ones can be used as useful acylating agents for some *N*-nucleophiles. The method represents an alternative for the synthesis of the antifungal agent salicylanilide. Regioselectivity in the Schmidt reaction on fused cycloalkanone ring of some pyran-2-one derivatives was investigated. It was found that isomers ratio is mostly affected by altering the temperature of reaction mixture. A new heterocyclic system, pyrano[3,2-*b*]azepine was prepared by the application of the Schmidt reaction . An investigation of transformations of pyran-2-one derivatives containing an amino group on positon 3 with hydrazine hydrate into 1,4-dihydropyridazines was performed. It was shown that different starting pyran-2-one derivatives required specific conditions for the successful reaction towards 1,4-dihydropyridazine derivatives with carbohydrazido group on position 3. A mechanism of these transformations was postulated on the basis of some isolated intermediates. The carbohydrazides were further transformed into the corresponding esters by the application of various inorganic oxidants (thallium(III) nitrate trihydrate, copper(II) acetate hydrate, ceric(IV) ammonium nitrate). Depending on the amount and type of the oxidant used in the reaction an oxidation of a 1,4-dihydropyridazine ring also occured.

Citosolna vloga cisteinskih proteaz papainove naddružine sesalskega izvora

J. Rozman Pungerčar
Institut Jožef Stefan, Ljubljana, Slovenija
Mentor: V. Turk
Somentor: B. Turk
Institut Jožef Stefan, Ljubljana, Slovenija

Prisotnost lizosomskih cisteinskih proteaz (LCP) v citosolu je povezana s številnimi patološkimi stanji, kot so apoptoza in vnetna obolenja. Nekatere LCP ostanejo v citosolu dalj časa aktivne in stabilne. Na primeru papaina in katepsina B smo pokazali, da k njihovi stabilnosti pomembno prispevajo ionske interakcije, od katerih je najpomembnejša interakcija med katalitskima ostankoma Cys25 in His159, ki tvorita ionski par. Poleg aktivnih LCP so izven lizosomov zasledili tudi njihove domnevno neaktivne proencimske oblike. Na primeru prokatepsina B smo pokazali, da so tudi proencimi lahko katalitsko aktivni. Prokatepsin B namreč hidrolizira sintetični substrat Z-Arg-Arg-AMC, njegovo delovanje pa inhibirajo sintetični inhibitor cisteinskih proteaz, E-64, in njegovi analogi. Raziskave o sodelovanju LCP v apoptoznih in vnetnih procesih vključujejo uporabo visoko specifičnih inhibitorjev in substratov. Ugotovili smo, da so substrati in inhibitorji LCP ter substrati kaspaz zadovoljivo specifični, medtem ko sintetični inhibitorji kaspaz učinkovito inhibirajo tudi cisteinske proteaze papainove naddružine. S kinetičnimi meritvami smo določili konstante hitrosti inaktivacije (k_2/K_1: 1-355000 $s^{-1}M^{-1}$) ter konstante inhibicije (K_i: 0.5-40 μM) cisteinskih proteaz papainove naddružine s kaspaznimi inhibitorji. Poleg tega smo pokazali, da fluorometilketonski inhibitorji kaspaz skoraj popolnoma inhibirajo (70–95 %) aktivnost LCP v kulturah sesalskih celic Jurkat in HEK 293T v koncentracijah, ki se uporabljajo pri študiju apoptoznih procesov.

Cytosolic Role of Mammalian Papain-like Cysteine Proteases

J. Rozman Pungerčar
Jozef Stefan Institute, Ljubljana, Slovenia
Supervisor: V. Turk
Co-supervisor: B. Turk
Jozef Stefan Institute, Ljubljana, Slovenia

Lysosomal cysteine proteases (LCP) are also found outside lysosomes especially at some pathological states, including apoptosis and inflammation. In the cytosol several LCP retain their stability and activity for certain period of time. Using papain and cathepsin B as the model enzymes we have shown that ionic interactions are very important for the stability of papain-like cysteine proteases, among these the most important being the active site Cys-His ion pair. Besides mature LCP there exist their presumably inactive zymogens in the cytosol as well. We have been able to show by various methods using procathepsin B that also zymogens possess small activity. Procathepsin B was thus shown to hydrolyze synthetic substrate Z-Arg-Arg-AMC. Furthermore, this proenzyme was also inhibited by synthetic cysteine protease inhibitor E-64 and its analogues. The role of LCP and caspases in apoptotic and inflammation processes has been investigated in the presence of different synthetic inhibitors and substrates. We showed that substrates and inhibitors of LCP and caspase substrates were sufficiently specific, while caspase inhibitors were nonselective, inhibiting also papain-like cysteine proteases. Inactivation rate constants (k_2/K_1: 1-355000 $s^{-1}M^{-1}$) and inhibition constants (K_i: 0.5-40 μM) of LCP by caspase inhibitors were determined by kinetic measurements. Cell-permeable fluoromethylketones also efficiently blocked activity (70–95 %) of LCP in Jurkat and HEK 293T cell cultures at inhibitor concentrations widely used in studies of apoptosis.

Sinteza in dinamično obnašanje integrirane mreže destilacijskih kolon

G. Sobočan
Fakulteta za kemijo in kemijsko tehnologijo, Univerza v Mariboru, Slovenija
Mentor: P. Glavič
Fakulteta za kemijo in kemijsko tehnologijo, Univerza v Mariboru, Slovenija

Prikazana je izboljšana sintezna metoda za ugotavljanje ekonomsko najugodnejše strukture toplotno integriranega večkolonskega destilacijskega sistema. Metoda sloni na računalniški simulaciji procesa, termodinamski metodi uščipa ter uporabi enostavnih in natančnih modelov destilacijskih kolon v sistemih ločevanja mešanice petih oz. šestih komponent. Osnovo ideje predstavlja integrabilnostni kriterij minimiranja intrakolonskih ploščin, s katerim ločuje zaporedja destilacijskih kolon glede na njihovo zmožnost toplotne integracije. Kot najboljše kolone so se pokazale tiste z najmanjšo znotrajkolonsko ploščino. Ugotovljeno je bilo, da so ekonomsko najugodnejši toplotno neintegrirani in integrirani sistemi tudi dinamično stabilni. Ob dobrem načrtovanju regulacije je odziv na motnje v pretoku in sestavi napajalne mešanice znotraj sprejemljivih meja.

Synthesis and Dynamic Behaviour of Distillation Columns Integrated Network

G. Sobočan
Faculty of Chemistry and Chemical Engineering, University of Maribor, Slovenia
Supervisor: P. Glavič
Faculty of Chemistry and Chemical Engineering, University of Maribor, Slovenia

An improved synthesis method to find economically most favourable structure of heat integrated distillation train system is presented. The method is based on the computational simulation of the process combined with pinch analysis. Short-cut or rigorous models of distillation columns can be used in the systems for separation of five and six component mixtures. The main idea is the integrability criterion for minimisation of intracolumn areas, which defines optimal distillation columns sequences with regard to their ability for heat integration. As the best columns arrangements those with the smallest intracolumn area were found. It was confirmed that the economically most promising heat integrated and nonitegrated systems are stable in dynamic way too. With a good control design, disturbances in feed flow rate and composition are in acceptable limits.

Enrofloksacin - metaanaliza učinkovitosti zdravljenja obolenj pri domačih živalih

L. Ščuka
Krka, d. d., Novo mesto, Slovenija
Mentor: P. Lazar
Veterinarska fakulteta, Univerza v Ljubljani, Slovenija

Metaanaliza omogoča raziskovalcem, da združujejo rezultate in dognanja več študij. V pregled smo izbrali 110 študij o zdravstvenem varstvu prašičev, 67 za prežvekovalce in 60 za perutnino, skupno 237 študij. Pregledali in ovrednotili smo učinkovitost zdravljenja z enrofloksacinom in občutljivost *in vitro* bakterij za enrofloksacin. V sistematičnem pregledu smo lahko primerjali učinkovitost enrofloksacina in drugih protimikrobnih zdravil. Naredili smo 19 metaanaliz, v 7 primerih pa smo izračunali posamezno velikost učinka za določen parameter. Iz rezultatov je razvidno, da je enrofloksacin močno učinkovit za zdravljenje okužb dihal pri vseh domačih živalih ($P < 0,01$). Enrofloksacin je zelo učinkovit za zdravljenje vseh okužb s kolibakterijo in salmonelami pri prašičih in perutnini ($P < 0,001$), medtem ko bi bilo treba za kolibacilozo goveda opraviti dodatne klinične študije, kar velja tudi za salmonelozo. Ob upoštevanju vseh izsledkov se je enrofloksacin pokazal za učinkovitega v zdravljenju mikoplazmoz pri perutnini in prašičih, za govedo pa bi bile potrebne še dodatne raziskave. Da je dajanje enrofloksacina učinkovito, smo z metaanalizo pri perutnini ugotovili za pasterelozo pri puranih, kužno korico, stafilokokozo in okužbo z *R. anatipestifer* pri racah ($P < 0,001$). Pri prašičih je bilo zdravljenje z enrofloksacinom v poskusni skupini značilno učinkovitejše od kontrolne za sindrom MMA ($P = 0,002$), okužbe urinarnega trakta ($P < 0,05$) in okužbe s streptokoki ($P = 0,045$). Za Glässerjevo bolezen razlike s kontrolno skupino niso bile značilne ($P = 0,25$), a je povročitelj (*H. parasuis*, $n = 124$) v 100 % občutljiv za enrofloksacin. Za sajavost pujskov obstaja izredno velika občutljivost *in vitro S. hyicus* za enrofloksacin (98,3 %, $n = 744$). Za pojasnjenje vseh vprašanj o mastitisu pri govedu bi morali opraviti eno ali več študij, v katerih bi uporabili enrofloksacin, saj naši izsledki kažejo, da ni učinkovitejši od kontrolne skupine ($P = 0,79$). Rezultati o občutljivosti povzročiteljev mastitisa za enrofloksacin *in vitro* pa so dobri. Tudi za zdravljenje endometritisa pri govedu bi morali narediti še kakšen klinični poskus, saj razlike med poskusno in kontrolno skupino niso bile značilno različne ($P = 0,49$), čeprav so bili rezultati v korist zdravljenja z enrofloksacinom.

Enrofloxacin – the Meta-Analysis of the Efficacy of Disease Treatment for Domestic Animals

L. Ščuka
Krka, d. d., Novo mesto, Slovenia
Supervisor: P. Lazar
Veterinary Faculty, University of Ljubljana, Slovenia

Meta-analysis provides a combination of the results and findings obtained in various studies. For a closer review, we chose 110 studies on health care in pigs, 67 in ruminants and 60 in poultry, i.e. 237 studies. We reviewed and evaluated the efficacy of the treatment of various infections with enrofloxacin and bacterial *in vitro* susceptibility to enrofloxacin. In a systematic review, we compared efficacy of enrofloxacin and other antimicrobial agents. We prepared 19 meta-analyses, while in 7 cases we also calculated the individual effect size for a specific parameter. It is evident from the results that enrofloxacin is potently effective in the treatment of respiratory infections in all domestic animals ($P < 0.01$). Enrofloxacin is very effective in the treatment of all coli and salmonella infections in pigs and poultry ($P < 0.001$), while additional studies about colibacillosis and salmonellosis in cattle would be necessary. After taking into account all findings, it was revealed that enrofloxacin is effective in the treatment of mycoplasma infections in poultry and pigs, while additional studies would be necessary in cattle. A meta-analysis in poultry revealed that administration of enrofloxacin is effective in pasteurellosis in turkeys, and in infectious coryza, staphylococcosis and *R. anatipestifer* infection in ducks ($P < 0.001$). In pigs, treatment with enrofloxacin was significantly more effective in the trial group that in the control group for MMA syndrome ($P = 0.002$), urinary tract infections ($P < 0.05$) and streptococcal infections

(P =0.045). For Glässer's disease the difference, in comparison to the control group was not significant (P = 0.25), however, the pathogen (*H. parasuis*, n = 124) was 100% susceptible to enrofloxacin. In greasy pig disease, there is a high *in vitro* susceptibility of *S. hyicus* to enrofloxacin (98.3 %, n = 744). To be able to answer to the complex questions about mastitis in cattle, one or more additional studies with enrofloxacin would be necessary, as our results indicate that enrofloxacin is not more effective than drugs in control groups. However, the *in vitro* results on susceptibility of mastitis pathogens to enrofloxacin are good. An additional study would also be necessary for the treatment of endometritis in cattle, since the difference between the trial and the control group was not statistically significant (P = 0.9), although the results were in favour of the treatment with enrofloxacin.

Modeliranje vzdolžnih skrčkov in prečnih deformacij skeletnih mišic

B. Šimunič
Fakulteta za elektrotehniko, Univerza v Ljubljani, Slovenija
Mentor: V. Valenčič
Fakulteta za elektrotehniko, Univerza v Ljubljani, Slovenija

Razvoj sodobne računalniške opreme, modernih elektronskih naprav in senzorjev je v zadnjih letih omogočil velik napredek k lažjim raziskavam področja biomehanike. Hiter razvoj opreme je sprožil potrebe po tovrstnih raziskavah in tako se v zadnjem času vedno več športnikov, njihovih trenerjev in fizioterapevtov ter bolnikov z živčno mišičnimi obolenji poslužuje raznih metod za preventivo in kurativo poškodb ter dimenzioniranja trenažnega in rehabilitacijskega procesa.
Na Fakulteti za elektrotehniko v Ljubljani poteka razvoj neinvazivne in selektivne tenziomiografske merilne metode, ki spremlja odziv mišice v prečni smeri glede na smer delovanja sile mišice. S hkratno uporabo tenziomiografske metode in metode merjenja navora v sklepu, smo modelirali povezanost odzivov mišic izmerjenih v prečni in longitudinalni smeri delovanja mišične sile. Pri interpretaciji rezultatov izmerjenih v *in vivo* pogojih na človeku smo si pomagali z vzporedno raziskavo na izolirani mišici krastače (*bufo bufo*). V *in vitro* pogojih smo lahko opravili bolj nadzirane poskuse, brez večine motečih dejavnikov, ki vplivajo na meritve v *in vivo* pogojih.
Ugotovili smo, da je dinamika odzivov odmika trebuha mišice različna od dinamike navora v sklepu. Vzroke moramo iskati v motečih dejavnikih vzdolžnega odziva kot so: trenje ob okolišnja tkiva, dušenje mišičnih ovojnic, mehanika sklepa, vpliv debeline maščevja in kože nad trebuhom ter elastična ter dušilna komponenta tetiv. Glede na naštete dejavnike smo razložili vpliv njih na intrinzične lastnosti mehanike mišičnega krčenja ter z modeliranjem podali glavne razloge razlikovanja med obema smerema mišičnega odzivanja.

Modeling of Longitudinal Shortening and Transversal Deformation of Skeletal Muscle

B. Šimunič
Faculty of Electrical Engineering, University of Ljubljana, Slovenia
Supervisor: V. Valenčič
Faculty of Electrical Engineering, University of Ljubljana, Slovenia

Development of computer capacity, technologically modern electronic devices and sensors has in recent years enabled greater technological progress in the field of biomechanical researches. Modern technology has launched more scientific approach of athletes training process as well as rehabilitation process. Therefore many athletes, trainers and physiotherapists incorporate new findings for prevention and rehabilitation of injuries, training and rehabilitation dimensioning.
A new non-invasive and selective method called Tensiomyography for monitoring skeletal muscle properties in transversal direction was developed at Faculty of electrical engineering in Ljubljana. On the basis of results obtained with simultaneous use of Tensiomyographic method torque measuring method we calculated mathematical models in order to present either similarity or heterogeneity of these results. Standard characteristics of muscle activation elicited by electrical stimulation were measured and interpreted by results obtained with in vitro measurements of toad gastrocnemius muscle. *In vitro* experiment allowed us to perform measurement on isolated muscle with and without its tendon. The study revealed that dynamics of transversal muscle belly displacement and longitudinal muscle joint torque differs due to disturbing parameters in longitudinal directions, such as: friction of surrounding tissue, restriction of muscle belly enlargement by fascia, joint mechanics, influence of the skin fold and tendons. With modeling approach we interpreted influences of disturbing parameters on intrinsic properties on mechanics of muscle contraction.

Signalna pot odziva HOG na povečano slanost pri halofilni črni kvasovki *Hortaea werneckii*

M. Turk
Medicinska fakulteta, Univerza v Ljubljani, Slovenija
Mentorica: A. Plemenitaš
Medicinska fakulteta, Univerza v Ljubljani, Slovenija

Hortaea werneckii je halofilna črna kvasovka, ki spada med askomicetne glive. Značilno zanjo je, da tolerira zelo visoke koncentracije soli v svojem okolju in raste celo v nasičeni raztopini NaCl. V okviru doktorskega dela smo preučevali, ali podobna signalna pot HOG, ki se pri *S. cerevisiae* odziva na povišano slanost v okolju, obstaja tudi pri *H. werneckii*. Dokazali smo prisotnost komponent signalne poti HOG, homologov MAPK Hog1p in njej nadrejene kinaze Pbs2p, pri *H. werneckii*. Predlagali smo model delovanja MAPK HwHog1p in MAPKK HwPbs2p pri *H. werneckii* pri različnih pogojih slanosti. Izolirali in analizirali smo gen za ključno MAPK signalne poti HOG pri *H. werneckii*, *HwHOG1*, in ugotovili, da obstaja ena sama kopija tega gena ter da na njegovo izražanje slanostni stres ne vpliva. Iz nukleotidnega zaporedja smo izvedli aminokislinsko zaporedje kinaze HwHog1p. Ugotovili smo, da je aktivnost HwHog1p uravnavana na posttranslacijski ravni. Funkcijo kinaze HwHog1p smo preverili s komplementacijo mutanta *S. cerevisiae hog1*Δ z genom *HwHOG1* in pokazali, da HwHog1p v *S. cerevisiae* nadomesti Hog1p le delno. Postavili smo strukturni model encima v nefosforilirani in fosforilirani obliki in ga uvrstili v poddružino glivnih s stresom aktiviranih protein-kinaz (YSAPK). Čeprav sta aminokislinsko zaporedje in modelna struktura HwHog1p zelo podobna ostalim homologom Hog1p, pa različna lokacija fosforilirane in nefosforilirane oblike kinaze HwHog1p, podaljšana aktivacija poti HOG pri *H. werneckii* in le delna komplementacija funkcije Hog1p v *S. cerevisiae* kažejo na različno uravnavanje signalne poti HOG pri halofilni kvasovki *H. werneckii* v primerjavi z organizmi, ki niso prilagojeni na visoke slanosti.

HOG Signaling Pathway in Halophilic Black Yeast *Hortaea werneckii*

M. Turk
Faculty of Medicine, University of Ljubljana, Slovenia
Supervisor: A. Plemenitaš
Faculty of Medicine, University of Ljubljana, Slovenia

Hortaea werneckii (*Dothideales*, *Ascomycota*) is a halophilic melanized yeast-like fungal species, a member of an arbitrary group of so-called black yeasts. It can tolerate extremely high salt concentrations in its environment and can grow at salinities ranging from 0% to a saturated solution of NaCl (32% NaCl (w/v)). In the present study we investigated whether the hyperosmotic signaling pathway similar to HOG pathway in *S. cerevisiae* exists in *H. werneckii*. We demonstrated the presence of MAPK homologue Hog1p and its upstream kinase Pbs2p in *H. werneckii*. The hypothetical model of interactions among HwHog1p and HwPbs2p under different NaCl concentrations was proposed. A homologue of the HOG1 gene, *HwHOG1*, was isolated and analysed. The existence of only one copy of the *HwHOG1* gene in the genome of *H. werneckii* was observed. Its activation by increased salinity is regulated at the post-translational level. We checked the function of kinase HwHog1p with complementation of *S. cerevisiae hog1*Δ mutant and found that HwHog1p can only partially replace Hog1p. A structural model of kinase HwHog1p in phosphorylated and dephosphorylated form was established. On the basis of aminoacid sequence similarity with fungal homologues of Hog1p, HwHog1p was classified into a subgroup of fungal stress-activated protein kinases (SAPKs). Although the aminoacid sequence and the model structure of HwHog1p were similar to other Hog1p homologues, different localization of phosphorylated and dephosphorylated form of HwHog1p, the prolonged activation of HOG pathway in *H. werneckii* under stress conditions and only partial complementation of *S. cerevisiae* Hog1p function point to a different regulation of HOG signaling pathway in halophilic *H. werneckii* in comparison to other organisms not adapted to high salinities.

Vloga oblike p41 invariantne verige in cistatina C pri uravnavanju cisteinskih proteaz v antigen predstavitvenih celicah

V. Zavašnik Bergant
Institut Jožef Stefan, Ljubljana, Slovenija
Mentor: J. Kos
Krka, d. d., Novo mesto, Slovenija
Biotehniška fakulteta, Univerza v Ljubljani, Slovenija

Aktivacija limfocitov T CD4+ z antigen predstavitvenimi celicami (APC) je nujno potrebna za učinkovit specifičen imunski odziv na antigen. APC proteolitsko razgradijo antigen in ga s pomočjo molekul MHC II predstavijo na svoji površini receptorjem limfocitov T. Uravnavanje proteolitske aktivnosti z endogenimi inhibitorji proteaz ima pomembno vlogo pri procesiranju in predstavitvi antigena, pri čemer sta bila v dosedanjih raziskavah na živalskih modelih kot možna inhibitorja katepsinov L in S predlagana šaperonska molekula oblika p41 invariantne verige ter nizko molekularni inhibitor cistatin C. Za določitev invariantne verige v humanih bioloških vzorcih smo pripravili in ovrednotili dve različni monoklonski protitelesi: prvo, ki spozna tiropinu podoben inhibitorni fragment na obliki p41 invariantne verige, in drugo, ki spozna epitop na neinhibitornem lumenskem delu invariantne verige oblik p41 in p31. Preučili smo porazdelitev in vsebnost oblik p31 in p41 in cistatina C ter kolokalizacijo s katepsini S, L in H v bezgavkah, v pljučnem tkivu in tumorjih, v izoliranih monocitih in iz njih diferenciranih nezrelih in zrelih (aktiviranih) dendritičnih celicah ter v promonocitni celični liniji U937. Uporabili smo imunohistokemijsko analizo, konfokalno fluorescenčno mikroskopijo, pretočno citometrijo, prenos po Westernu, ELISA teste in elektronsko mikroskopijo. Naši rezultati kažejo na vlogo humane oblike p41 invariantne verige pri uravnavanju delovanja katepsinov L in H v makrofagih v germinativnih središčih in v manjši populaciji sinusnih makrofagov v bezgavki ter v alveolarnih makrofagih v pljučih. Ne potrjujejo pa regulatorne vloge cistatina C pri uravnavanju encimske aktivnosti katepsinov S, L in H med zorenjem humanih dendritičnih celic.

Role of p41 Isoform of Invariant Chain and Cystatin C in Regulating Cysteine Proteases in Antigen-presenting Cells

V. Zavašnik Bergant
Jožef Stefan Institute, Ljubljana, Slovenia
Supervisor: J. Kos
Krka, d. d., Novo mesto, Slovenia
Biotechnical Faculty, University of Ljubljana, Slovenia

Activation of CD4+ T cells by antigen-presenting cells (APC) is an obligatory step in the efficient adaptive immune response to an antigen. Antigen is processed and presented on the surface of APC in complex with MHC class II molecules for recognition by T cell receptors. The regulation of proteolytic activity of cysteine proteases by their endogenous inhibitors plays an important role in modulating antigen presentation. Using animal models, a chaperone molecule p41 isoform of invariant chain and low molecular weight inhibitor cystatin C were suggested to modulate the proteolytic capacity of cysteine proteases cathepsins L and S. In order to study the invariant chain in human biological samples, two different monoclonal antibodies were prepared and characterized: first, recognising inhibitory thyropin-like p41 fragment on the p41 isoform of invariant chain, and second, recognising an epitope on the non-inhibitory lumenal portion of p41 and p31 isoforms of invariant chain. The distribution and content of p31 and p41 isoforms and cystatin C was defined and co-localization with cathepsins S, L and H determined and their possible interactions addressed in human lymph nodes, lung tissue and tumours, isolated monocytes and differentiated immature and mature (activated) dendritic cells and in the promonocyte U937 cell line. Immunohistochemical analysis, confocal fluorescence microscopy, flow cytometry, Western blotting, ELISA and electron microscopy were applied. Our results confirmed the possible regulatory role of human p41 isoform of invariant chain on cathepsins L and H in tingible body macrophages inside germinal centres and in the minor population of sinus-lining macrophages in lymph nodes and in alveolar macrophages in lungs. On the contrary, we have rejected the hypothesis on the regulatory role of cystatin C on cathepsin S, L and H during human dendritic cell maturation.

33.
Krkine
nagrade
33rd
Krka
Prizes

3 Krka Prize Winners´ Poster Session

Vloga znotrajceličnih zank receptorja za glukagonu podobni peptid-1 pri prenosu signala

A. Bavec
Medicinska fakulteta, Univerza v Ljubljani, Slovenija
Mentor: M. Zorko
Medicinska fakulteta, Univerza v Ljubljani, Slovenija

Prejšnje študije so odkrile pomen tretje znotrajcelične zanke receptorja za glukagonu podobni peptid-1 (RGP-1) pri prenosu signala na G_s in G_{i1} heterotrimerne G-proteine. Na osnovi napovedi terciarne strukture podganjega RGP-1 (Swiss-Prot server) in teoretičnega tridimenzionalnega modela RGP-1 smo naredili sintetične peptide, ki so bili izvedeni iz treh znotrajceličnih zank podganjega RGP-1. Sintetične peptide znotrajcelične zanke 1 (peptid ZZ1), znotrajcelične zanke 2 (peptid ZZ2) in znotrajcelične zanke 3 (peptid ZZ3) smo testirali z različnimi vrstami heterotrimernih G-proteinov. Kot model za proučevanje aktivnosti G-proteinov smo uporabili celice sf9, v katere smo s pomočjo Baculovirusov vnesli gen z zapisom za podenoto α_s, α_{i1}, α_{11} in α_0 ter gen za podenoto $\beta_1\gamma_2$ heterotrimernih G-proteinov. Peptid ZZ3 je stimuliral vse vrste testiranih G-proteinov, medtem ko sta peptida ZZ1 in ZZ2 različno učinkovala na G-proteine iz membran celic sf9. Peptida ZZ1 in ZZ2 sta stimulirala heterotrimerne G-proteine G_s in modulirala učinek peptida ZZ3. Peptida ZZ1 in ZZ2 nista imela učinka na G-proteine G_0. Peptid ZZ1 ni učinkoval na G_{i1} in G_{11}, medtem ko je peptid ZZ2 stimuliral omenjene G-proteine in moduliral učinek peptida ZZ3. Peptid ZZ3 torej predstavlja glavno stikalo pri prenosu signala na G-proteine, medtem ko sta peptida ZZ1 in ZZ2 modulatorja v signalni poti, ki sta pomembna pri razlikovanju različnih vrst G-proteinov. Sprememba v sekundarni strukturi peptida ZZ3 je sovpadala s spremembo aktivnosti peptida ZZ3 na G-proteine. Peptidu ZZ3 se je pri višjih mikromolarnih koncentracijah povečal delež b-strukture, ki je tako spremenil svojo strukturo iz neaktivne v aktivno konformacijo ter stimuliral delovanje G-proteinov G_s, G_0, G_{i1} in G_{11}. Povečan delež b-strukture pa lahko tudi pomeni, da je peptid ZZ3 agregiral v oligomerne komplekse, npr. dimere. Molekularni model dimera RGP-1 je nakazal, da se zaradi tesnega pakiranja ZZ3 v receptorju (podobno agregaciji peptida ZZ3) lahko poveča delež b-strukture v dimeru, kar vodi v aktivno stanje receptorja, ki stimulira G-proteine. Peptid ZZ3 je inhibiral mono-ADP-ribozilacijo podenote b heterotrimernih G-proteinov v plazemskih membranah iz celic ovarija kitajskega hrčka, najverjetneje tako, da je učinkoval kompetativno skupaj s podenoto b na endogeno mono-ADP-riboziltransferazo. Možno pa je tudi, da je šlo pri mono-ADP-ribozilaciji peptida ZZ3 za neodvisen encimski proces, ki ni povezan z inhibicijo mono-ADP-riboziltransferazne aktivnosti. V proces inhibicije mono-ADP-ribozilacije podenote b s peptidom ZZ3 na pertusis toksin občutljivi G-proteini niso bili vključeni. Ker smo pri naši študiji kot orodje za proučevanje interakcij receptor-G-protein uporabili sintetične peptide, ki so izvedeni iz prve, druge in tretje znotrajcelične zanke RGP-1, bi bilo mogoče izsledke te naloge koristno uporabiti pri sintezi novih zdravil za zdravljenje diabetesa mellitusa tipa 2.

Different Role of Intracellular Loops of Glucagon-like Peptide-1 Receptor in Signal Transduction

A. Bavec
Faculty of Medicine, University of Ljubljana, Slovenia
Supervisor: M. Zorko
Faculty of Medicine, University of Ljubljana, Slovenia

Previous studies revealed the importance of the third intracellular loop of glucagon-like peptide-1 receptor (GLP-1R) in coupling to G_s and G_{i1} proteins. In order to further study the signaling mechanisms of GLP-1R, we tested three peptides, corresponding to the sequences of the first (IC_1), the second (IC_2), and the third (IC_3) intracellular loop of GLP-1R, for their interactions with heterotrimeric G-proteins of different types ($G_{\alpha s}$, $G_{\alpha o}$, $G_{\alpha i1}$, and $G_{\alpha 11}$ plus $\beta_1\gamma_2$) overexpressed in sf9 cells. IC_3 peptide powerfully stimulates all types of tested G-proteins whereas IC_1 and IC_2 peptides show differential effects on G proteins. Both, IC_1 and IC_2 peptides activate G_s and cooperate with IC_3 peptide in its stimulation. G_o is not affected by IC_1 and IC_2. G_{i1} and G_{11} are not affected by IC_1 but are activated by IC_2, which in activation cooperates with IC_3. We suggest that GLP-1R is not coupled only to G_s and G_{i1}, but also to G_o and G_{11}.

IC_3 loop is the main switch that mediates signaling via GLP-1R to G-proteins, while IC_1 and IC_2 loops are important in discrimination between different types of G-proteins. Structural changes of IC_3 peptide in its secondary structure correlate with its effect on G-proteins. IC_3 peptide adopts much less β-structure at lower than at higher micromolar concentration. It would be possible that at higher concentrations the IC_3 structure is changed from "switch off" to "switch on" conformation that stimulates G_s, G_o, G_{i1} and G_{11}-proteins. Additionally, increase of the amount of β-structure might also reflect the aggregation of IC_3 peptide at higher concentrations into a oligomeric structures such as dimers. Molecular model of GLP-1 dimer revealed the possible interaction of monomers in the region of IC_3 loops. Interaction between IC_3 loops could increase the amount of β-structure in GLP-1 dimer, which leads in its active conformation in coupling with G-proteins. Additionally, IC_3 peptide inhibited mono-ADP-ribosylation of β-subunit of heterotrimeric G-proteins mediated by endogenous mono-ADP-ribosyltransferase in plasma membrane from Chinese hamster ovary cells. It is possible that peptide IC_3 acts as competitive substrate for endogenous mono-ADP-ribosyltransferase in such way that it reduces mono-ADP-ribosylation of β-subunit. Alternatively, mono-ADP-ribosylation of IC_3 peptide which is mediated by endogenous mono-ADP-ribosyltransferase could be independent enzymatic process. Inhibition of mono-ADP-ribosylation of β-subunit by IC_3 peptide is not mediated via pertussis toxin sensetive G-proteins. Sinthetic peptides derived from the first, the second and the third intracellular loop of GLP-1R had been used as a tool for studying receptor-G-protein interaction in this study. Therefore, it is possible that our results could be usefully used in the development of new peptide drugs for treatment of diabetes mellitus type 2.

Za pravilno interpretacijo od cAMP-odvisne transaktivacije je pomembna izbira normalizacijskega vektorja: primer človeškega gena *CYP51*

J. Ačimovič
Medicinska fakulteta, Univerza v Ljubljani, Slovenija
Mentorica: M. Fink
Somentorica: D. Rozman
Medicinska fakulteta, Univerza v Ljubljani, Slovenija

CYP51 je holesterogeni gen, njegovo izražanje pa uravnavata od cAMP in od sterolov odvisna signalna pot, ki sta karakterizirani z transkripcijskimi faktorji CREM in CREB oz. SREBP. PKA pa sodeluje v signalni poti cAMP. Pri svojem delu smo poskušali ugotoviti ali PKA vpliva tudi na SREBP posredovano prepisovanje človeškega gena *CYP51*. Ugotovili smo, da k SREBP-1a in -1c dodana PKA poveča njuno aktivnost, medtem ko je pri SREBP-2 in -2gc aktivnost v prisotnosti/odsotnosti PKA nespremenjena. To je v skladu s predhodnimi študijami s *CYP51-CAT* poročevalskim sistemom, kjer so ugotovili, da je za aktivnost SREBP-1a verjetno odgovorna fosforilirana oblika. Zanimiva ugotovitev je, da PKA ob prisotnosti SREBP-2 ne vpliva na prepisovanje *CYP51*. Presenetljiva ugotovitev pa je, da PKA ne aktivira s CREM in CREB posredovanega prepisovanja *CYP51*. Pri svojem delu smo poizkušali v laboratorij vpeljati tudi nov poročevalski sistem luciferaze in pri tem smo ugotovili, da vsi priporočeni normalizacijski vektorji (promotor timidinske kinaze) niso optimalni, ker se odzivajo na preiskovane dražljaje in tako lahko prekrijejo odziv preiskovanega promotorja.

Selection of the Normalization Vector is Crucial for Correct Interpretation of the cAMP-dependent Transactivation: Example of the Human *CYP51* Gene

J. Ačimovič
Faculty of Medicine, University of Ljubljana, Slovenia
Supervisor: M. Fink
Co-supervisor: D. Rozman
Faculty of Medicine, University of Ljubljana, Slovenia

CYP51 is a cholesterogenic gene. Its transcription is regulated with cAMP-dependent and sterol-dependent signaling pathway which are characterized with CREM and CREB transcription factors or SREBP. PKA participates in cAMP signaling pathway. The aim of our work was to find out if PKA affects SREBP mediated signaling pathway of human *CYP51* gene transcription. The results demonstrated that transactivation potential of SREBP-1a and -1c on the CYP51 promoter is increased when PKA is expressed, while SREBP-2 and -2gc activity remain unaltered in the presence/absence of PKA. These results confirm our previous studies by the *CYP51-CAT* reporter system, where PKA-dependent phosphorylation increased the trans-activation potential of SREBP-1a. Interestingly, PKA does not influence the transcription of *CYP51* by SREBP-2. We have also tried to establish whether the new luciferase reporter system into the lab and we have found out that all reporter vectors (promotor timidin kinase) are not optimal, because they are responsive to the tested agents stimuli and so they can cover the response of the tested promotor.

Uporaba pravil dobre laboratorijske prakse v laboratorijih za preizkušanje zdravil

S. Barič
Krka, d. d., Novo mesto, Slovenija
Mentorica: M. Murn
Krka, d. d., Novo mesto, Slovenija
Somentorica: M. Sollner Dolenc
Fakulteta za farmacijo, Univerza v Ljubljani, Slovenija

V tovarni zdravil Krka v Novem mestu opravljajo določene predklinične (toksikološke) študije lastnih zdravil namenjenih za humano in veterinarsko uporabo. Tovrstne študije za ugotavljanje varnosti opravljajo v skladu z mednarodno sprejetimi navodili in standardi. Eden od osnovnih standardov, ki zagotavlja kakovost in verodostojnost predkliničnih študij, so tudi Načela dobre laboratorijske prakse. V Krki (Služba za predklinične raziskave) so julija 2001 pridobili »Potrdilo o skladnosti z načeli dobre laboratorijske prakse« za toksikološke in mutagenične študije. Za pridobitev »Potrdila« so morali opraviti določene, dokaj obsežne aktivnosti in pripraviti določeno dokumentacijo. Opravila sem pogovore in intervjuje z vsemi zaposlenimi, ki so me seznanili z aktivnostmi med pripravami za dosledno in neprekinjeno izvajanje Načel dobre laboratorijske prakse pri rednem delu ter za pregled laboratorijev od strani pooblaščenega inšpektorja R Slovenije. Ugotovila sem, da so za uvedbo načel dobre laboratorijske prakse potrebovali nekaj let intenzivnega dela, izobraževanja, ter nekaj organizacijskih sprememb in gradbenih posegov. Z leti so ustvarili skupino ljudi z ustrezno strokovno in tehnično izobrazbo.
Vsi zaposleni so mnenja, da je opravljanje dela v skladu z načeli dobre laboratorijske prakse mogoče le v njihovem medsebojnem sodelovanju in sodelovanju z vodstvom in osebjem za zagotavljanje kakovosti. Zavedajo se tudi, da le dosledno izvajanje načel dobre laboratorijske prakse in neprekinjeno dograjevanje sistema zagotavlja kvaliteto opravljenega dela.

Good Laboratory Practice in Preclinical Evaluation of Drugs

S. Barič
Krka, d. d., Novo mesto, Slovenia
Supervisor: M. Murn
Krka, d. d., Novo mesto, Slovenia
Co-supervisor: M. Sollner Dolenc
Faculty of Pharmacy, University of Ljubljana, Slovenia

Pharmaceutical factory Krka in Novo mesto performed some of preclinical (toxicological) studies of drugs intended for human and veterinary use. These studies are used for safety evaluation and are performed in accordance to internationally accepted guidelines and standards. One of the most important standards is Good laboratory practice guidelines, which give an assurance of high quality and credibility of the study. Department for Preclinical Research, Research and Development Division in Krka have got Good Laboratory Practice certificate for toxicological and mutagenicity studies in July 2001. I performed interviews with staff in Toxicological Department and they acquainted me with activities needed to implement GLP Guideline in every day's work as well as with activities needed for the inspection, done by the official inspector of Republic of Slovenia. I have got information that extensive activities have been done during several last years to get ready for consistent and constant implementation of GLP guidelines. These activities included education of staff, certain changes in organisation and building reconstructions. They have a team of adequately educated people (experts and technicians), which have clearly defined job description and responsibilities.
The staff believed that consistent implementation of GLP Guidelines into their every day's work is possible only, if cooperation exist within team, with management and QA. The also believed that consistent implementation of GLP Guidelines as well as continuous "building" of the system is the warranty for the quality.

Sinteza sintonov za pripravo antagonistov integrinskih receptorjev

M. Breže
Fakulteta za farmacijo, Univerza v Ljubljani, Slovenija
Mentorica: M. Sollner Dolenc
Somentor: M. Anderluh
Fakulteta za farmacijo, Univerza v Ljubljani, Slovenija

Razvoj antagonistov različnih podtipov integrinskih receptorjev pomeni napredek na širokem terapevtskem področju in izboljšanje dodedanje terapije zlasti pri zdravljenju miokardnega infarkta, restenoze po posegu PTCA, preprečevanju napadov angine pectoris ter zdravljenju rakavih obolenj, osteoporoze in nekaterih avtoimunih obolenj. V terapevtski uporabi že zasledimo antagoniste fibrinogenskega ($\alpha_{IIb}\beta_3$) receptorja, v fazi kliničnih in predkliničnih raziskav pa so tudi spojine, ki modulirajo delovanje $\alpha_V\beta_3$ $\alpha_V\beta_5$, $\alpha_5\beta_1$, $\alpha_4\beta_1$ in $\alpha_4\beta_7$ receptorjev.
Endogeni ligand za nekatere integrinske receptorje je tripeptidno aminokislinsko zaporedje RGD (arginil-glicil-aparaginska kislina), na osnovi katere razvijajo selektivne in učinkovitejše predvsem antagoniste za željen tip receptorja.
Z namenom ugotoviti ali 7-amino-2-aminometil-2,4-dimetil-3-okso-3,4-dihidro-2*H*-1,4-benzoksazin in 7-amino-2-aminometil-2,4-dimetil-3,4-dihidro-2*H*-1,4-benzoksazin lahko služita kot mimetika dela RGD-aminokislinskega zaporedja, smo raziskali sintezne poti za pripravo teh spojin. Raziskali smo tudi možnost vpeljave benzamidinskega fragmenta in derivatov malonske kisline v vlogi posameznih farmakofornih skupin, ki posnemajo bodisi farmakoforni del arginina ali asparaginske kisline.
Pripravljeni sintoni bodo zanimivi gradniki predvsem za sintezo selektivnih antagonistov vitronektinskega receptorja.

Synthesis of Synthons for Preparation of Integrin Receptor Antagonists

M. Breže
Faculty of Pharmacy, University of Ljubljana, Slovenia
Supervisor: M. Sollner Dolenc
Co-supervisor: M. Anderluh
Faculty of Pharmacy, University of Ljubljana, Slovenia

The development of different subtypes of integrin receptor antagonists brings the progress on a wide therapeutic field that includes treatment of myocardial infarction, restenosis after PTCA, prevention of angina pectoris and treatment of cancer, osteoporosis and several autoimune diseases. Some of $\alpha_{IIb}\beta_3$ integrin antagonists have already found their place in the therapy. Moreover, compounds which modulate activity of $\alpha_v\beta_3$, $\alpha_V\beta_5$, $\alpha_5\beta_1$, $\alpha_4\beta_1$ in $\alpha_4\beta_7$ integrin receptors have already entered in the phase of preclinical and clinical development.
Endogenous ligands for some integrin receptors share the same binding motif- sequence of three amino acids RGD (arginyl-glycin-asparaginic acid). RGD sequence represents the basis for development of selective and effective mostly antagonists of different integrin receptors.
Our main intention was to determine whether 7-amino-2-aminomethyl-2,4-dimethyl-3-oxo-3,4-dihydro-2-*H*-1,4-benzoxazine and 7-amino-2-aminomethyl-2,4-dimethyl-3,4-dihydro-2-*H*-1,4-benzoxazine can serve as RGD sequence mimetics. Our interest was also to research the possibility of introducing a benzamidine fragment and derivatives of malonamic acid as pharmacophore groups, which mimic either the pharmacophore part of arginine or aparaginic acid.
The prepared synthons represent interesting building units useful in synthesis of selective vitronectin receptor-antagonists.

Vpliv povezovalnih zank in kationov na tvorbo gvaninskih kvadrupleksov

M. Cevec
Fakulteta za kemijo in kemijsko tehnologijo, Univerza v Ljubljani, Slovenija
Mentor: M. Polak
Somentor: J. Plavec
Kemijski inštitut, Ljubljana, Slovenija

Z gvanini bogata DNK zaporedja se nahajajo v številnih regijah evkariontskega kromosoma, vključno s telomerami. Lahko tvorijo strukture višjega reda (npr. gvaninske kvadruplekse), ki imajo pomembno vlogo pri replikaciji kromosoma, genski regulaciji in mejozi. Pripravili smo tri DNK oligonukleotide z zaporedjem tipa d(G_4-zanka-G_4). Zanko so sestavljali abazični in aciklični preostanki. Zanimalo nas je, kako na tvorbo G-kvadrupleksov vplivajo različne zanke in različni monovalentni kationi (K^+, Na^+ in NH_4^+). Oligonukleotide smo pripravili s pomočjo avtomatizirane sinteze na trdnem nosilcu, njihovo zvijanje smo ob titraciji s solmi spremljali z opazovanjem karakterističnih resonanc v ^{1}H NMR spektrih. Ugotovili smo, da imajo vsi trije modificirani oligonukleotidi ob prisotnosti enakega iona v imino območju protonskega spektra signale pri enakem kemijskem premiku. NMR spektre študiranih G-kvadrupleksov smo primerjali z NMR spektri zaporedja d($G_4T_4G_4$), katerega struktura je poznana. Na osnovi rezultatov merjenja translacijskih difuzijskih koeficientov smo sklepali, da so pred titriranjem oligonukleotidi v enoverižni obliki, po titriranju pa v dimerni G-kvadrupleksni obliki. 3D strukturo smo študirali s pomočjo 1D in 2D homo- in hetero-nuklearnih NMR eksperimentov. V NOESY spektrih smo opazili nekaj gvaninov v *sin* konformacijah, iz česar sklepamo, da so oligonukleotidi v raztopini zviti v "fold-back" G-kvadrupleksno strukturo z antiparalelnimi verigami.

Influence of Different Loop Regions and Cations on Guanine Quadruplex Formation

M. Cevec
Faculty of Chemistry and Chemical Technology, University of Ljubljana, Slovenia
Supervisor: M. Polak
Co-supervisor: J. Plavec
National Institute of Chemistry, Ljubljana, Slovenia

Guanine-rich DNA sequences are found in various regions of eukaryotic chromosomes, including telomeres, and can form higher order structures (e.g. guanine quadruplexes), which are thought to have important roles in chromosome replication, gene regulation and meiosis. In the present work three DNA oligonucleotides with common sequence d(G_4-loop-G_4) were prepared. The loops consisted of abasic and acyclic residues. The influence of different loop regions on guanine quadruplex formation was studied as a function of the addition of either K^+, Na^+ and NH_4^+ ions. DNA oligonucleotides were synthesized on a solid support using nucleic acid synthesizer. The structure and conformation of DNA oligonucleotides was studied using NMR spectroscopy (1D and 2D homo- and hetero-nuclear experiments) and diffusion experiments. NMR spectra were compared with NMR spectra of sequence d($G_4T_4G_4$), structure of which was already determined. The diffusion experiments have shown that DNA oligonucleotides before titration adopt a single stranded structure and after titration bimolecular structure. The examination of NOESY spectra clearly established some guanine residues with *syn* conformation, which means, that fold-back G-quadruplex structures with antiparalel strands were formed.

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

P. Cvelbar
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

Kompleksi platine so potencialni citostatiki, zato so raziskave kompleksov platine izrednega pomena tako za medicino kot tudi za farmacevtsko industrijo. Prva taka citostatika sta bila kompleksa cisplatina in karboplatina. Oba sta najbolj uporabljena citostatika na svetu, vendar povzročata hude stranske učinke in delujeta le na določene tumorne celice. Tako so nekateri tumorji nanju odporni, pri drugih pa povzročata razvoj odpornosti tumornih celic. Zaradi veliko neželenih stranskih učinkov obstaja interes po razvoju novih kompleksov platine s širšim spektrom delovanja, izboljšano klinično učinkovitostjo in boljšo topnostjo.
Sintetizirali smo komplekse Pt(II) z različnimi aminskimi ligandi: izopropilamin, 2,2'-bipiridil, 3-hidroksimetilpiridin in 2,2-aminometilpropanol. Pri tem smo dobili naslednje spojine: ($[PtI_2(ipa)_2]$, $[PtI_2(ipa)]_2$, $[PtI_2(ipa)(NH_3)]$, $[PtI_2(3hmpy)(ipa)]$, $[PtI_2(3hmpy)_2]$, $[PtI_2(bipy)]$, $[PtI_2(bipy)(NO_3)]$, $[PtI_2(amp)_2]$ in $[PtI(amp)_2]$.
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 and Characterization of Platinum(II) Complexes with Various Amine Ligands

P. Cvelbar
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 exert potential antitumor activity, therefore they are interesting for both medicine and pharmaceutical industry. The first drugs with antitumor activity were cisplatin and carboplatin. They are the most widely used antitumor drugs in the world. However they cause severe side effects and act only on some forms of tumors. Some tumors have natural resistance while others develop resistance after the initial treatment. Because of these disadvantages, there is a continuous need for new platinum drugs with broader spectrum of activity, better clinical efficacy and better solubility.
We synthesized Pt(II) complexes with various amine ligands: isopropylamine, 2,2'-bipyridine, 3-hydroxymethylpiperidine, 2,2-aminomethylpropanol and obtained the following complexes: ($[PtI_2(ipa)_2]$, $[PtI_2(ipa)]_2$, $[PtI_2(ipa)(NH_3)]$, $[PtI_2(3hmpy)(ipa)]$, $[PtI_2(3hmpy)_2]$, $[PtI_2(bipy)]$, $[PtI_2(bipy)(NO_3)]$, $[PtI_2(amp)_2]$ and $[PtI(amp)_2]$.
After the successful syntheses we optimised the reaction conditions in order to get pure products with an optimal yield. We controlled the course of reactions, identified the products, coordination sphere and purity of products by means of X-ray powder analysis, IR spectroscopy, ^{1}H and ^{195}Pt NMR spectroscopy and thermogravimetrical analysis.

Paralelna sinteza na osnovi metil 2-acetilamino-3-dimetilaminopropenoata

P. Čebašek
Fakulteta za kemijo in kemijsko tehnologijo, Univerza v Ljubljani, Slovenija
Mentor: J. Svete
Fakulteta za kemijo in kemijsko tehnologijo, Univerza v Ljubljani, Slovenija

Alkil 2-substituirani-3-dimetilaminopropenoati ter njihovi ciklični in kiralni analogi so se izkazali za enostavno dostopna in izredno raznolika sintezna orodja pri pripravi različnih heterocikličnih sistemov in aminokislinskih derivatov. Po strukturi najpreprostejšega med njimi, metil 2-acetilamino-3-dimetilaminopropenoat, smo uporabili pri preučevanju pretvorb z različnimi aromatskimi in heteroaromatskimi amini v danes vse bolj pomembni kombinatorni sintezi tako v raztopini kot tudi na trdni fazi. Literaturno že poznani sintezni pristop k reakcijam, pri katerih poteka izmenjava dimetilaminske skupine propenoatov z amini kot *N*-nukleofili, smo prilagodili za paralelno sintezo v raztopini in na ta način pripravili metil 2-acetilamino-3-arilaminopropenoate in metil 2-acetilamino-3-[(5-nitropiridin-2-il)amino]propenoat kot derivate α,β-nenasičenih-α-aminokislin. Paralelno sintezo z metil 2-acetilamino-3-dimetilaminopropenoatom smo razširili še na reakcije na polimernem nosilcu, kjer smo iskali optimalne pogoje za vezavo omenjenega reagenta na Wangov polimerni nosilec, pogoje za izmenjavo dimetilaminske skupine imobiliziranega reagenta s heterocikličnimi amini ter v zadnji stopnji pogoje za kondenzacijo obročnega dušikovega atoma amina z estrsko skupino propenoata, kjer hkrati pride tudi do cepitve vezi med sintetiziranim derivatom pirimidona in Wangovim polimernim nosilcem.

Parallel Synthesis Based on Methyl 2-acetylamino-3-dimethylaminopropenoate

P. Čebašek
Faculty of Chemistry and Chemical Technology, University of Ljubljana, Slovenia
Supervisor: J. Svete
Faculty of Chemistry and Chemical Technology, University of Ljubljana, Slovenia

Alkyl 2-substituted-3-dimethylaminopropenoates and their cyclic and chiral analogues are easily available and versaitile reagents for the preparation of a variety of heterocyclic systems and amino acid derivatives. One of the most simple among them, methyl 2-acetylamino-3-dimethylaminopropenoate, was investigated in regard to its transformations with various aromatic and heteroaromatic amines in the combinatorial chemistry approach in solution and solid phase. The synthetic approach described in the literature for the dimethylamino group substitution in propenoates with amines as *N*-nucleophiles was adopted for the parallel synthesis in the solution phase. Methyl 2-acetylamino-3-arylaminopropenoate and methyl 2-acetylamino-3-[(5-nitropyridin-2-yl)amino]propenoate, as α,β-unsaturated amino acid derivatives, were thus prepared. The parallel synthesis with methyl 2-acetylamino-3-dimethylaminopropenate was also extended to the solid-phase reactions using Wang resin. Optimal loading conditions for the attachment of the reagent to the Wang resin as well as reaction conditions for the substitution reactions and subsequent condensations were investigated.

Izolacija in lastnosti ksilanaze XynT iz vampne bakterije *Pseudobutyrivibrio xylanivorans* Mz5^T

T. Čepeljnik
Biotehniška fakulteta, Univerza v Ljubljani, Slovenija
Mentorica: R. Marinšek Logar
Biotehniška fakulteta, Univerza v Ljubljani, Slovenija

Pseudobutyrivibrio xylanivorans Mz5^T je vampna bakterija z zelo aktivnim encimskim sistemom za razgradnjo ksilana. Sestavlja ga več elektroforetsko različnih ksilanaz z molekulskimi masami od 30 do 146 kDa. Zanimiva je predvsem najmanjša, ki predstavlja večino aktivnosti v poznejših fazah mikrobne rasti. S postopnim obarjanjem in tekočinsko kromatografijo na hidrofobnih nosilcih nam jo je uspelo izolirati do elektroforetske čistosti in smo jo poimenovali XynT. Izolirana ksilanaza ima molekulsko maso 30 ± 0,8 kDa in se izraža v dveh različicah. Glavnina ima izoelektrično točko 5,08 ± 0,1, manjši del proteinov pa 5,9 ± 0,08. Mikroheterogenost je lahko posledica dveh genov ali pa posttranslacijskih modifikacij. Ksilanaza XynT razgrajuje le ksilan iz različnih virov (najaktivnejše ksilan ovsenih plev, manj aktivno pa brezov in bukov ksilan), ne pa tudi drugih bolj ali manj podobnih polisaharidov. Prav tako ne more odcepljati substituent, ki so vezane na osnovno verigo. Ksilan razgradi do večjih oligosaharidov. Je torej prava endoksilanaza, ki je najaktivnejša pri 38° C in vrednosti pH = 5,6. Na osnovi teh dejstev smo ksilanazo XynT uvrstili v družino 11 glikozil-hidrolaz, kar smo potrdili tudi s primerjanjem pridobljenega aminokislinskega zaporedja na amino-koncu proteina. S pridobljenim znanjem smo razširili poznavanje ksilanolitičnega encimskega sistema te bakterije in predvideli vlogo ksilanaze XynT pri razgradnji ksilana.

Isolation and Characterisation of Xylanase XynT from Rumen Bacterium *Pseudobutyrivibrio xylanivorans* Mz5^T

T. Čepeljnik
Biotechnical Faculty, University of Ljubljana, Slovenia
Supervisor: R. Marinšek Logar
Biotechnical Faculty, University of Ljubljana, Slovenia

Pseudobutyrivibrio xylanivorans Mz5^T is a rumen bacterium with a very potent enzyme system for xylan degradation. The system is composed of many electrophoretically distinct xylanases with molecular weights ranging from 30 to 146 kDa. The most interesting is the smallest enzyme that represents the majority of xylanolytic activity in the later growth phases. We were able to isolate it to electrophoretic homogeneity by fractionate precipitation and hydrophobic interaction chromatography. Isolated xylanase was named XynT and its molecular weight was 30 ± 0.8 kDa. Native electrophoresis and isoelectric focusing revealed its microheterogeneity resulting in the majority of the proteins with pI of 5.08 ± 0,1 and minority with pI of 5.9 ± 0.8. Two isoenzymes could be the result of two genes or posttranslational modifications. Xylanase XynT is able to degrade only xylan from different sources (it is most active towards oat spelts xylan, and less active is towards birchwood or beechwood xylan) and no other more or less similar polysaccharides. Niether it can cleave the substituents from the xylan backbone. It degrades xylan into bigger oligosaccharides, so it is a true endoxylanase. The most active is at 38° C and pH 5.6. Its position in the family 11 of glycosilhydrolases was confirmed by amino acid sequence, too. These results have expanded the knowledge of the xylanolytic enzyme system of *Pseudobutyrivibrio xylanivorans* Mz5^T and propose the role of xylanase XynT in the xylan degradation.

Farmakoekonomska analiza različnih zdravljenj osteoporoze

B. Džajkovska
Fakulteta za farmacijo, Univerza v Ljubljani, Slovenija
Mentor: A. Mrhar
Fakulteta za farmacijo, Univerza v Ljubljani, Slovenija

Osteoporoza je resen javnozdravstveni in družbeni problem zaradi visoke prevalence med starajočo se populacijo ter visokih stroškov, ki jih povzroča zdravljenje le-te kot tudi zdravljenje njenih posledic (zlomi). Glavni namen te naloge je bil izvesti farmakoekonomsko analizo za vse vrste zdravljenja osteoporoze, da bi opredelili in primerjali učinkovitost različnih zdravljenj z namenom optimiranja učinkovitosti zdravljenja osteoporoze. Retrospektivna modelna analiza stroškovne učinkovitosti in stroškovne uporabnosti je vključevala štiri starostne skupine pomenopavzalnih žensk in naslednje možnosti zdravljenja: brez terapije, alendronat, etidronat, risedronat, kalcitonin, hormonsko nadomestno zdravljenje (HNZ) in raloksifen. Analiza je bila izvedena z Markovim modelom na podlagi slovenskih podatkov o stroških in verjetnostih pojava posameznih dogodkov ter podatkov o klinični učinkovitosti zdravil iz objavljenih meta analiz. Rezultati analize stroškovne učinkovitosti in stroškovne uporabnosti kažejo, da sta alendronat in HNZ stroškovno najbolj učinkoviti zdravili pri preprečevanju zlomov zaradi osteoporoze za vse starostne skupine pomenopavzalnih žensk. Na podlagi rezultatov naloge lahko sklepamo, da bo širše uveljavljanje farmakoekonomskih analiz in principov omogočilo učinkovitejše preprečevanje osteoporotičnih zlomov in večjo kakovost življenja osteoporotičnih žensk ter hkrati optimizacijo stroškov v sistemu zdravstvenega varstva.

Pharmacoeconomic Analysis of Different Drug Treatments for Osteoporosis

B. Džajkovska
Faculty of Pharmacy, University of Ljubljana, Slovenia
Supervisor: A. Mrhar
Faculty of Pharmacy, University of Ljubljana, Slovenia

Osteoporosis is a chronic disease with serious socioeconomic impact because of its high prevalence and enormous costs for treatment of this condition and its consequences, i.e. fractures. Main objective of this study was to perform a pharmacoeconomic analysis from societal perspective, with the aim to evaluate and compare all available treatments for osteoporosis in order to optimize the efficiency of osteoporosis treatment. A retrospective, modeled cost-effectiveness and cost-utility analysis included postmenopausal women divided in four age groups, and the following treatment options: no drug therapy, alendronate, etidronate, risedronate, calcitonin, hormone replacement therapy (HRT), and raloxifene. The analysis was performed on the basis of a Markov model developed with using data on probabilities and costs derived from Slovenian sources, and data on clinical efficacy pooled from meta-analyses. Results of our cost-effectiveness and cost-utility analysis found alendronate and HRT as the most cost-effective treatment options for prevention of osteoporotic fractures in all age groups of postmenopausal women. The results of our study suggest that the application of pharmacoeconomic analysis can optimize the efficiency of osteoporosis treatment, improve the quality of life for patients and improve the efficiency of the healthcare system.

Priprava arilakrilatnih polimernih nosilcev in uporaba pri sintezi heterocikličnih spojin

V. Frankovič
Pedagoška fakulteta, Univerza v Mariboru, Slovenija
Mentor: P. Krajnc
Fakulteta za kemijo in kemijsko tehnologijo, Univerza v Mariboru, Slovenija

Z uporabo vodne suspenzijske polimerizacije smo sintetizirali netopne kopoli(stiren-p-nitrofenilakrilatne) nosilce s 5% in 20% zamreženjem ter razmerjem akrilat/stiren 1/1. Ob uporabi propelerskega mešala in 2L reaktorja smo pri 750 obr/min z radikalsko polimerizacijo dobili polimerne nosilce v obliki kroglic premera med 50-150 μm. Kot monomere smo uporabili 4-nitrofenilakrilat, stiren in divinilbenzen, kot površinsko aktivno snov polivinilpirolidon ter kot iniciator azobisizobutironitril. Esterske skupine obeh nosilcev smo pretvorili najprej do kisline in nato še do kislinskega klorida. Z analizno metodo za določanje vsebnosti klorida smo ugotavljali obstojnost kislinskega klorida v daljšem časovnem obdobju. Ugotovili smo, da kislinski klorid, ki je imobiliziran na 20% zamreženem nosilcu, ne izgubi aktivnosti pri shrambi pri –18^{0}C po 1 mesecu, medtem ko kislinski klorid, imobiliziran na 5% zamreženem nosilcu, pod enakimi pogoji, izgubi približno 30% aktivnosti. Imobilizirani kislinski klorid smo uporabili za reakcijo z metilnim estrom glicina in dobili glicinmetilesterski derivat, ki smo ga v nadaljevanju s *t*-butoksibis(dimetilamino) metanom pod inertnimi pogoji v suhem toluenu pretvorili v 3-diaminopropenoatni derivat. Na polimerni 3-diaminopropenoatni derivat smo vezali 2-aminopiridin in dobili imobilizirano biciklično spojino.

Preparation of Arylacrylate Polymer Supports And Their Use in the Synthesis of Heterocyclic Compounds

V. Frankovič
Pedagogical Faculty, University of Maribor, Slovenia
Supervisior: P. Krajnc
Faculty of Chemistry and Chemical Engineering, University of Maribor, Slovenia

We synthesised unsoluble poly(styrene-co-p-nitrophenylacrylate) supports with 5% and 20% crosslinking density and acrylate/styrene ratio 1/1 using water suspension polymerisation. Insoluble polymer supports in the form of beads with diameter 50-150 μm were obtained using a propeller tipe overhead stirrer at 750 rpm. This was obtained by radical initiation. 4-nitrophenylacrylate, styrene and divinylbenzene were used as monomers, polyvinylpyrrolidone as a surface active substance and azobisisobutyronitrile as an initiator. Ester groups of both supports were transformed into acid and afterwards into acid chloride. Stability of immobilised acid chloride in one month period was studied by chlorine content determination. 20% crosslinked immobilised acid chloride showed no diminishing of activity after one month storage at –18^{0}C while 5% crosslinked immobilised acid chloride lost 30% of activity. From polymer supported acid chloride a glycinmethylester derivative was prepared in high yield and was transformed into a 3-diaminopropenoate derivative using *t*-butoxybis(dimethylamino)methane under inert atmosphere and in dry toluene. Subsequently 2-aminopyridine was bound to the 3-diaminopropenoate derivative resulting in a polymer supported bicyclic compound.

Določevanje organokositrovih spojin v vodi s plinsko kromatografijo

J. Jakše
Zavod za zdravstveno varstvo, Novo mesto, Slovenija
Mentorica: L. Zupančič–Kralj
Fakulteta za kemijo in kemijsko tehnologijo, Univerza v Ljubljani, Slovenija
Somentorica: N. Šoštarić-Virc
Zavod za zdravstveno varstvo, Novo mesto, Slovenija

Prva organokositrova spojina je bila sintetizirana leta 1852. Njihova industrijska uporaba pa se je začela šele leta 1940 z industrijo plastike. Dandanes so to zelo široko uporabljene spojine, saj se uporabljajo na zelo različnih področjih; kot stabilizatorji v industriji plastike, kot antioksidanti, biocidi, fungicidi, insekticidi, uporabljajo pa se tudi kot dodatek k barvi za premaz ladij.
Zaradi velike toksičnosti teh spojin je bil namen mojega dela uvesti DIN standard za določanje organokositrovih spojin v odpadnih vodah ter podtalnici.
Organokositrove spojine sem določevala z GC/MS analizo. Najprej sem naredila umeritvene krivulje za sedem organokositrovih spojin. V vodo sem dodala določeno množino organokositrove spojine, uravnala pH z acetatnim pufrom, nato pa dodala derivatizacijsko sredstvo natrijev tetraetilborat. Derivat sem nato iz raztopine ekstrahirala s heksanom, ekstrakt uparila na manjšo količino, ter ga analizirala z GC/MS. Pri določanju organokositrovih spojin v odpadnih vodah pa je bilo potrebno tako dobljeni ekstrakt še nadalje čistiti na silikagelski koloni, ter ga šele nato analizirati. Analiza ekstraktov je pokazala, da na naklon umeritvene krivulje vpliva učinkovitost derivatizacije in ekstrakcije, čiščenje ekstrakta pa tudi ionizacija v MS detektorju.
Metoda, opisana v DIN standardu, se je izkazala kot zanesljiva, vendar bi jo bilo potrebno nekoliko optimirati, da bi bil čas analize nekoliko krajši.

Determination of Organotin Compounds in Water with Gas Chromatography

J. Jakše
Institute of Public Health, Novo mesto, Slovenia
Supervisior: L. Zupančič–Kralj
Faculty of Chemistry and Chemical Technology, University of Ljubljana, Slovenia
Co-supervisor: N. Šoštarić-Virc
Institute of Public Health, Novo mesto, Slovenia

First organotin compound was synthesized in 1852. The use of organotin compounds started in plastic industry in 1940. Nowadays these compounds are widely used in many different areas: as stabilizers in plastic industry, as biocydes, fungicides, insecticides and as antifouling paints for ships.
In my work I wanted to introduce DIN standard for determination organotin compounds in waste waters and underground waters.
I used GC/MS analysis for determination of organotin compounds. I made calibration curves for seven organotin compounds. I put into water different quantities of organotin compounds, set pH with acetate solution, and added sodium tetraethylborate. I used extraction with hexane to get derivate out of solution. I reduced extract to smaller quantity and analysed it with GC/MS method. Determination of organotin compounds in waste waters and the analysis of extract were possible only after cleaning it through the silicagel. Analysis of extract has shown how derivatisation and extraction, cleaning of extract and ionisation in this detector influence the calibration curves.
The method described in DIN standard appeared to be reliable. However it needs some optimisation; the time of analysis should be a bit shorter.

Primerjava aktivosti H^+-ATPaz pri dveh sevih glive *Aspergillus niger*

E. Jambrošič
Kemijski inštitut, Ljubljana, Slovenija
Mentor: M. Legiša
Kemijski inštitut, Ljubljana, Slovenija

Delo je bilo opravljeno na industrijsko pomembnem mikroorganizmu, glivi *Aspergillus niger*, ki je delovni organizem pri proizvodnji primarnih in sekundarnih metabolitov.
Plazma membranske H^+- ATPaze gliv so protonske črpalke, ki sodelujejo pri vzpostavitvi in vzdrževanju elektrokemijskega gradienta protonov. Imajo pomembno vlogo pri vnosu hranil v celico in pri regulaciji intracelularnega pH, ta pa vpliva na fiziologijo celice, kar se kaže tudi v hitrosti njene rasti. Zaradi različnih izmerjenih intracelularnih pH vrednosti pri dveh sevih glive *A. niger* smo želeli preveriti aktivnost njunih H^+- ATPaz. Zato smo oba seva glive gojili v prisotnosti amonijevega nitrata in glutaminske kisline ter pri tem zasledovali hitrost padca ekstracelularnega pH in naraščanje biomase. Ugotovili smo, da je pri bolj produktivnem sevu A60 glede izločanja citronske kisline, aktivnost H^+-ATPaze nižja, kar očitno povzroči padec intracelularne pH vrednosti, to pa mora vplivati na celoten metabolizem in fiziologijo glive. Izkazalo se je, da ima encim pri obeh sevih enako afiniteto do substrata ter različna pH optimuma in različne maksimalne encimske hitrosti.
Z *in vitro* dodanimi V^{5+} ioni smo ugotovili različno občutljivost H^+- ATPaz obeh sevov na orto-vanadat. Podobno smo ugotovili tudi za Cu^{2+} ione. Ko smo vanadijeve in bakrove ione dodali v gojišče, se je izkazalo, da zaviralno vplivajo na nivo ekspresije H^+-ATPaz.

Comparison of H^+-ATPase Activities among two *Aspergillus niger* Strains

E. Jambrošič
National Institute of Chemistry, Ljubljana, Slovenia
Supervisor: M. Legiša
National Institute of Chemistry, Ljubljana, Slovenia

The work has been done on a biotechnologically important microorganism, filamentous fungus *Aspergillus niger*, which is able to excrete large quantities of primary (citric acid) as well as secondary metabolites.
Plasma membrane H^+-ATPases in fungi are proton pumps which participate in the foundation and maintenance of the electrochemical gradient of protons across the membrane. They play an important role at the transport of nutrients into the cell and at the regulation of intracellular pH, which might influence the metabolism and concomitantly the specific growth rate. Because of different intracellular pH values detected in two *A. niger* strains, as reported previously, we intended to verify the activity of their H^+-ATPases. Therefore, both fungal strains were cultivated in the presence of ammonium nitrate and glutamic acid. The drop of extracellular pH and the increase of biomass were followed. We stated that with more productive strain the measured enzyme was less active and unable to extrude protons fast enough to maintain pH homeostasis. The drop of intracellular pH value might result in changes of metabolism leading to higher productivity in regard to citric acid excretion. We showed that enzyme of both strains had the same affinity to substrate but different pH optimum and different maximum enzyme value.
The presence of V^{5+} ions in the measuring system revealed different sensibility of H^+-ATPases of both strains toward orto-vanadate. Similar results were obtained also for Cu^{2+} ions. When vanadium and copper ions were added into the medium, it turned out that they influenced the level of expression of H^+-ATPases.

Kristalna struktura kompleksa katepsina H s stefinom A

N-terminalni aminokislinski preostanki inhibitorja se lahko prilagodijo aktivnemu mestu endo- oz. eksopeptidaze

S. Jenko
Institut Jožef Stefan, Ljubljana, Slovenija
Mentor: D. Turk
Somentor: G. Gunčar
Institut Jožef Stefan, Ljubljana, Slovenija

Regulacijska vloga lizosomskih cisteinskih proteaz je tesno povezana z njihovimi inhibitorji, ki preprečujejo nezaželeno proteolizo in s tem patološko delovanje encima. Kinetične študije lizosomskih cisteinskih proteaz s proteinskimi inhibitorji stefini so pokazale tri velikostne rede slabše konstante inhibicije za eksopeptidaze (K_i=0.3-100 nM) v primerjavi z endopeptidazami (K_i=0.05-130 pM). Da bi raziskali strukturne spremembe, ki nastanejo ob vezavi proteinskega inhibitorja cistatinskega tipa na lizosomsko cisteinsko aminopeptidazo, smo kristalizirali svinjski katepsin H v kompleksu s človeškim rekombinantnim stefinom A v dveh kristalnih oblikah, ortorombski in monoklinski, z resolucijama 2.4 Å in 2.8 Å. Pri kompleksih katepsin H s stefinom A ter papaina s stefinom B gre za podoben način interakcij, vendar z nekaterimi odločilnimi razlikami. Stefin A se bolj tesno prilega katepsinu H kot stefin B papainu. Vezava stefina A na katepsin H, za razliko od stefina B na papain, povzroči strukturne spremembe tako na encimu, kot tudi na inhibitorju. N-terminalni del stefina A se ob vezavi na encim uviha, delno izpodrine mini verigo in poruši strukturo insercijske zanke (Lys155A-Asp155D) katepsina H. Predvidevamo da je vzpostavitev značilne vezavne geometrije pri papainu podobni lizosomski cisteinski proteazi in inhibitorju cistatinskega tipa, pri kompleksu papaina s stefinom B onemogočena, zaradi karboksimetiliranega cisteina v aktivnem mestu papaina.

Crystal Structure of Stefin A in Complex with Cathepsin H

N-terminal Residues of Inhibitors can adapt to the Active Sites of Endo- and Exopeptidases

S. Jenko
Jožef Stefan Institute, Ljubljana, Slovenia
Supervisor: D. Turk
Co-supervisor: G. Gunčar
Jožef Stefan Institute, Ljubljana, Slovenia

Endogenous protein inhibitors such as stefins and cystatins are one of the most important means of control of enzymatic activity of papain-like lysosomal cysteine proteases. Stefins inhibit endopeptidases in the picomolar range (K_i=0.05-130 pM) and exopeptidases in the nanomolar range (K_i=0.3-100nM). To investigate possible structural changes resulting from binding of a cystatin-type inhibitor to a lysosomal cysteine aminopeptidase, we have crystallized a complex of porcine cathepsin H with human stefin A, and determined the structure of the 1:1 stoichiometric complex in two crystal forms, orthorhombic and monoclinic, diffracting to 2.4 Å and 2.8 Å, respectively. The structures are similar to stefin B-papain complex, but with a few distinct differences. Binding of stefin A to cathepsin H is tighter than binding of stefin B to papain. The hairpin loops of stefin A structures lie about 0.8 Å closer to the bottom of the active site cleft than the hairpins of stefin B. The approach of stefin A to cathepsin H induces structural changes along the interaction surface of both molecules, whereas no such changes were observed in the stefin B-papain complex. On binding, the N-terminal residues of stefin A adopt the form of a hook, which pushes away cathepsin H mini-chain residues and distorts the structure of the short four residue insertion (Lys155A-Asp155D) unique to cathepsin H. The formation of the genuine binding geometry between a papain-like enzyme and a cystatin-type inhibitor as observed in cathepsin H-stefin A complex seems to be prevented by carboxymethylation of the active site cysteine of papain in papain-stefin B complex.

Izražanje sinteznega gena za brazein, sladki protein iz rastline *Pentadiplandra brazzeana*, v bakteriji *E. Coli*

Z. Jevnikar
Institut Jožef Stefan, Ljubljana, Slovenija
Mentor: B. Štrukelj
Fakulteta za farmacijo, Univerza v Ljubljani, Slovenija
Institut Jožef Stefan, Ljubljana, Slovenija
Somentorica: N. Slapar
Institut Jožef Stefan, Ljubljana, Slovenija

Brazein je protein sladkega okusa, izoliran iz plodov zahodnoafriške rastline *Pentadiplandra brazzeana*. V primerjavi z enako maso saharoze je približno 2000X bolj sladek, kar ga uvršča med doslej znanimi molekulami sladkega okusa v sam vrh. Brazein je termostabilen protein, saj ohrani sladek okus tudi po izpostavitvi temperaturi do 100°C. Stabilen je tudi v širokem območju pH in ima ugoden profil sladkega okusa. Molekula brazeina je majhna, kar olajša manipulacijo z genom. V današnjem času, ko je želja po zdravi in naravni prehrani vse večja, predstavlja brazein privlačno nadomestno sladilo za živilsko in farmacevtsko industrijo.
V okviru raziskovalnega dela smo najprej odpravili mutacijo, ki je nastala na sinteznem genu za brazein med njegovo pripravo. Mutacijo smo odstranili v verižni reakciji s polimerazo, kjer smo uporabili metodo z začetnim podaljšanim oligonukleotidom oz. »megaprimerjem«. Nato smo pripravili ekspresijski sistem za izražanje brazeina v periplazemski prostor *E. Coli*. Rekombinantni brazein bo osnova za nadaljnje biokemijske in biotehnološke raziskave.

Expression of the Synthetic Gene for Brazzein, a Sweet-tasting Protein from the Plant *Pentadiplandra brazzeana*, in *E. Coli*

Z. Jevnikar
Jožef Stefan Institute, Ljubljana, Slovenia
Supervisor: B. Štrukelj
Faculty of Pharmacy, University of Ljubljana, Slovenia
Jožef Stefan Institute, Ljubljana, Slovenia
Co-supervisor: N. Slapar
Jožef Stefan Institute, Ljubljana, Slovenia

Brazzein, a sweet-tasting protein, was originally isolated form the fruit of the West African plant *Pentadiplandra brazzeana*. Brazzein is 2000 times sweeter than sucrose on a weight basis, which places it at the top of the until now discovered sweet-tasting molecules. Brazzein was found to be very thermostable; its sweetnes remaines undiminished after heating to 100°C. It is also very pH-stable and has a favourable profile of sweet taste. The brazzein molecule is small, what makes manipulation with gene easier. The increasing demand for healthy and natural food product makes brazzein an attractive alternative sweetener to be used in food and pharmaceutical industry.
In our research work we successfully removed point mutation that emerged during the preparation of brazzein synthetic gene. We used polymerase chain reaction and »megaprimer« method. Then we prepared an expression system for the production of recombinant brazzein in the periplasm of *E. coli*. Recombinant brazzein will serve as a basis for further biochemical and biotechnological research.

Izražanje kaspaze-3 in survivina v hepatocelularnem karcinomu in v netumorskih jetrih ob tumorju

Č. Joksimović
Medicinska fakulteta, Univerza v Ljubljani, Slovenija
Mentor: A. Cör
Medicinska fakulteta, Univerza v Ljubljani, Slovenija

Apoptoza je programirana oblika celične smrti. Namen raziskave je bil ugotoviti delež apoptotskih celic v HCC v primerjavi z netumorskimi jetri ter vlogo kaspaze-3 v procesu apoptoze. V raziskavo smo vključili dvajset bolnikov s HCC. Z metodo TUNEL ter z imunohistokemičnim barvanjem na aktivno kaspazo-3 smo določili delež apoptotskih celic, izražen kot apoptotski indeks (AI). Z imunohistokemičnim barvanjem smo ugotovili izražanje prokaspaze-3 in survivina. AI določen po metodi TUNEL kot tudi z barvanjem na aktivno kaspazo je bil višji v HCC v primerjavi z netumorskimi jetri. Prav tako sta bila AI določena po obeh metodah višja v slabše diferenciranih HCC. V skupini HCC je bilo 75% primerov pozitivnih na prokaspazo-3. Ugotovili smo statistično značilno pozitivno povezanost izražanja prokaspaze-3 in aktivne kaspaze-3 v HCC. Na survivin je bilo pozitivnih 70% HCC. Ugotovili smo povečano izražanje survivina v HCC v primerjavi z netumorskimi jetri ter pozitivno povezanost izražanja survivina s stopnjo diferenciacije HCC. Statistično značilna je bila tudi pozitivna povezava med izražanjem survivina in aktivne kaspaze-3 v HCC. Sklepamo, da vzrok za nastanek in razvoj HCC ni zgolj izguba apoptotske aktivnosti hepatocitov, pač pa porušeno ravnovesje med apoptozo in celično proliferacijo. Pri tem ima survivin pomembno, vendar ne neposredno vlogo.

Caspase-3 and Survivin Expression in Hepatocellular Carcinoma and in Nontumorous Liver

Č. Joksimović
Faculty of Medicine, University of Ljubljana, Slovenia
Supervisor: A. Cör
Faculty of Medicine, University of Ljubljana, Slovenia

Apoptosis is programmed cell death. The percentage of apoptotic cells in HCC, compared to non-tumorous liver tissue was investigated. The role of caspase-3 was of great interest for this study. Twenty patients with HCC were included in the study. TUNEL and immunohistochemical staining for the detection of activated form of caspase-3 were used to determine the percentage of apoptotic cells (apoptotic index). Immunohistochemical staining was also performed for detection of procaspase-3 and survivin expression. Apoptotic index (AI) determined by TUNEL as well as by immunohistochemical staining for the detection of active form of caspase-3 was higher in HCC compared to non-tumorous liver. Both indices were also higher in less differentiated HCCs. Positive staining for procaspase-3 was present in 75% of HCC. Positive correlation between expression of procaspase-3 and activated form of caspase-3 proved to be statistically significant. Survivin positive reaction was detected in 70% of HCC. Stronger expression of survivin was detected in HCC compared to non-tumorous liver. Positive correlation between survivin expression and grade of tumor differentiation as well as significant association between survivin expression and active form of caspase-3 were found. These results indicate that the cause of HCC progression is not only the loss of apoptotic activity of hepatocytes, but mainly the imbalance between cell death and cell proliferation. Survivin has an important, but merely an indirect role in the process of HCC progression.

Razvoj in optimizacija metode preskusa raztapljanja z uporabo eksperimentalnih načrtov

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

V sklopu raziskovalne naloge smo z uporabo modernih statističnih tehnik študirali vplive različnih fizikalno kemijskih parametrov, ki vplivajo na sproščanje slabo topne zdravilne učinkovine iz tablet z lipofilnim ogrodnim sistemom. Kot modelno učinkovino smo uporabili diklofenak natrijevo sol (natrijeva sol 2-[(2,6-diklorofenil)amino] benzen ocetne kisline), kot modelni farmacevtski izdelek pa Naklofen® retard tablete s podaljšanim sproščanjem, ki vsebujejo 100 mg diklofenak natrijeve soli in tehnološko predstavljajo lipofilni ogrodni sistem. Najprej smo študirali vplive različnih fizikalno kemijskih parametrov na topnost zdravilne učinkovine. Nato smo z uporabo 2-nivojskega delnega eksperimentalnega načrta ugotavljali vplive šestih faktorjev na sproščanje zdravilne učinkovine iz tablet in določili signifikantne faktorje. S pomočjo metodologije odgovornih površin (Box-Behnkenovega eksperimentalnega načrta) smo s faktorji, ki so se izkazali za signifikantne, izdelali modelne funkcije, s katerimi lahko napovedujemo sproščanje diklofenak natrijeve soli v celotnem eksperimentalnem področju. Z uporabo eksperimentalnih načrtov smo v kratkem času in z relativno majhnim številom eksperimentov razvili in optimirali metodo sproščanja slabo topne zdravilne učinkovine iz tablet s podaljšanim sproščanjem.

Development and Optimization of Drug Release Method Using Experimental Design Methodology

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

The aim of our research was to apply modern statistical techniques (experimental designs) in the development and optimization of drug release methods. Diclofenac sodium (2-[(2,6-dichlorophenyl) amino]benzeneacetic acid monosodium salt) was selected as a model drug and Naklofen® retard prolonged release tablets, containing 100 mg of diclofenac sodium were chosen as a model lipophilic matrix system.
Solubility characteristics of diclofenac sodium in aqueous media with various ionic strengths, ionic compositions and pH in the range of 1 to 10 were determined. For estimation of effects of six different factors on the release of diclofenac sodium, 2-level fractional factorial design was applied. Box-Behnken experimental design is a response surface method, which was used to examine the relationship between four selected responses and significant factors and to provide second-order model functions for the whole experimental domain. Our study proved that experimental designs could efficiently be applied for characterization of analytical parameters affecting the drug release from prolonged release lipophilic matrix tablets and for development and optimization of drug release methods in similar formulations. It is an economical way of obtaining the maximum amount of information in a short period of time and with the fewest number of experiments.

Spremembe v genu *ATP2A2* v povezavi s tumorji glave in vratu, pljuč in širokega črevesa

B. Korošec
Medicinska fakulteta, Univerza v Ljubljani, Slovenija
Mentorica: M. Ravnik-Glavač
Medicinska fakulteta, Univerza v Ljubljani, Slovenija

Sprostitev kalcijevih ionov iz ER v citosol je ključna komponenta številnih signalnih povezav, ki nadzorujejo rast tumorskih celic, diferenciacijo ali apoptozo. Kalcijeve črpalke vrste SERCA, ki kopičijo kalcij v ER, igrajo pri tem pomembno vlogo. Številni proteini, vključeni v mehanizme nastanka raka, kot so proteini K-ras in Smad, so posredno povezani z nivojem kalcijevih ionov v celici, ki ga uravnavajo kalcijeve črpalke. Ta spoznanja in pa dejstvo, da so miške, haploinsuficientne za SERCA2, pogosto zbolevale za rakom, nas je spodbudilo, da smo želeli preveriti hipotezo o vključenosti in vlogi gena *ATP2A2* pri razvoju različnih vrst raka pri človeku. Z visoko občutljivimi metodami in tehnikami molekularnogenetske analize smo preučevali prisotnost sprememb v genu *ATP2A2* pri bolnikih z rakom glave in vratu, s pljučnim rakom in z rakom širokega črevesa in pri posameznikih brez raka. Celotno število novoodkritih spremenjenih alelov *ATP2A2* pri bolnikih s katerimkoli preučevanim rakom je bilo 22/574, medtem ko smo pri normalni kontrolni populaciji (248 alelov *ATP2A2*) ugotovili le eno od teh sprememb (χ^2 = 7,14, p = 0,01). Rezultati naših raziskav kažejo, da so spremembe v genu *ATP2A2* statistično signifikantno bolj pogoste pri bolnikih z rakom pljuč (6/190, χ^2 = 5,19, p = 0,02) in širokega črevesa (14/226, χ^2 = 12,94, p < 0,005), pogostejše, čeprav ne statistično signifikantno, pa tudi pri raku glave in vratu. Iz naših rezultatov lahko zaključimo, da obstaja povezava med napakami v genu *ATP2A2* in vsaj pljučnim rakom ter rakom širokega črevesa.

Alterations in *ATP2A2* Gene in Correlation with Head and Neck Tumours, Lung Tumours and Colon Tumours

B. Korošec
Faculty of Medicine, University of Ljubljana, Slovenia
Supervisor: M. Ravnik-Glavač
Faculty of Medicine, University of Ljubljana, Slovenia

Calcium release from endoplasmic reticulum in cytosol is a key component of numerous signal pathways controlling tumour growth, differentiation and cell death. Sequestration of Ca^{2+} in endoplasmic reticulum, from which it can be released during Ca^{2+} signaling events, is mediated by sarco(endo)plasmic reticulum Ca^{2+}- ATPazes (SERCAs) Numerous proteins involved in mechanisms of cancer development, like K-ras and Smads, depend on intracellular calcium level. These findings and the fact that SERCA haploinsuficient mice often developed cancer led us to examine the hypothesis about the involvement and the role of *ATP2A2* gene in human cancer development. We screened patients with head and neck carcinomas, lung cancer, colon cancer and unaffected individuals for genetic alterations in *ATP2A2* gene. The total number of novel altered alleles of all patients with different cancer was 22/574 while in unaffected (normal) population (248 alleles) we found only one of this alterations (χ^2 = 7,14, p = 0,01). Results of our research show that the changes in *ATP2A2* gene are statistically significantly more common in patients with lung cancer (6/190, (χ^2 = 5,19, p = 0,02) and colon cancer (14/226, χ^2 = 12,94, p < 0,005); the changes are common but not statistically significantly also in patients with head and neck carcinoma (2/158, χ^2 = 3,15, p = 0,08). We can conclude from our results that an association between alterations in *ATP2A2* gene and at least lung and colorectal cancer exists.

Določanje citotoksične aktivnosti *N*-substituiranih derivatov ftalimida in *N*-substituiranih derivatov glutaminske kisline

A. Kovač
Fakulteta za farmacijo, Univerza v Ljubljani, Slovenija
Mentor: S. Gobec
Fakulteta za farmacijo, Univerza v Ljubljani, Slovenija
Somentorica: M. Filipič
Nacionalni inštitut za biologijo, Univerza v Ljubljani, Slovenija

Maligna obolenja predstavljajo danes v razvitem svetu več kot 27% razlogov za smrt. Iskanje novih protitumornih učinkovin je torej za farmacevtsko industrijo izrednega pomena. Eden možnih pristopov k iskanju spojin vodnic s protitumornim delovanjem je rešetanje bank spojin naravnega in sinteznega izvora. Zaradi njihovega protitumornega delovanja v zadnjem desetletju zelo intenzivno raziskujejo derivate talidomida in glutaminske kisline. FDA je pred kratkim odobrila derivat ftalimida talidomid za zdravljenje različnih vrst tumorjev. Znano je tudi, da so mnoge spojine na osnovi ftalimida citotoksične *in vitro* proti mnogim tumorskim celičnim linijam in aktivne v *in vivo* protitumorskih preizkusih. Tudi derivati glutamina in glutaminske kisline so potencialne protitumorne učinkovine, ki lahko delujejo kot antagonisti glutamina in/ali folne kisline.
Iz banke spojin na Fakulteti za farmacijo smo izbrali dve seriji spojin: *N*-substituirane derivate ftalimida in *N*-substituirane derivate glutaminske kisline. Spojinam smo določili *in vitro* citotoksično delovanje na celice tumorja dojke (MDA-MB 231), karcinoma pluč (103H LCLC), jetrnega hepatoma (Hep G2) in na celično linijo normalnih epitelijskih celic (HUVEC). Eksponentno rastoče celice smo izpostavili spojinam (10^{-4} ali 10^{-5} M) in po 24 ter 48 urah določili citotoksičnost s kolorimetrično metodo (MTT test). Ugotovili smo, da kažejo nekateri lipofilni *N*-substituirani ftalimidi *in vitro* protitumorno delovanje v mikromolarnih koncentracijah.

Determination of Cytotoxic Activity of *N*-substituted Phthalimide and *N*-substituted Glutamic Acid Derivatives

A. Kovač
Faculty of Pharmacy, University of Ljubljana, Slovenia
Supervisor: S. Gobec
Faculty of Pharmacy, University of Ljubljana, Slovenia
Co-supervisor: M. Filipič
National Institute of Biology, Ljubljana, Slovenia

In the developed countries malignant diseases cause more than 27% of deaths. Consequently, development of new antitumor agents is a continuing interest of pharmaceutical industry. One of the possible approaches to new lead compounds discovery is screening of bank of compounds of both natural and synthetic origin. During the last decade thalidomide and glutamic acid derivatives have been investigated extensively due to their anticancer properties. FDA has recenty approved a phthalimide derivative thalidomide for treatment of different types of tumors. It is well known that many compounds with the phthalimide motif are either cytotoxic against the growth of human cancer cultured cell lines or active in *in vivo* antitumor assays. Glutamine and glutamic acid derivatives are also potential antitumor agents which may act through glutamine and/or folic acid antagonism.
From our inhouse bank of compounds we selected two series of compounds (phthalimide derivatives and glutamic acid derivatives) and screened them for their *in vitro* cytotoxic activity against three human tumor cell lines; MDA-MB 231 (breast cancer), 103H (large cell lung cancer), Hep G2 (hepatoma) and a normal cell line HUVEC (human endothelial cells). The exponentially growing cells were exposed to the compounds (10^{-4} ali 10^{-5} M) for 24 and 48 hours and cytotoxic activities were determined by a tetrazolium-based colorimetric assay (MTT assay). We found that some lipophilic *N*-substituted phthalimides show *in vitro* antitumor activity in micromolar concentrations.

Analiza neprevedenih delov evkariontskih genov *CYP51*

A. Krajnc
Biotehniška fakulteta, Univerza v Ljubljani, Slovenija
Mentorica: N. Debeljak
Somentorica: D. Rozman
Medicinska fakulteta, Univerza v Ljubljani, Slovenija

Gen *CYP51* kodira encim lanosterol 14α-demetilazo (*CYP51*), ki spada v naddružino citokromov P450 in sodeluje v biosintezi holesterola. Je eden najbolj ohranjenih genov *CYP* in je razširjen pri evkariontih in pri prokariontih. Naš namen je bil določiti nukleotidno zaporedje ter sklepati na lastnosti neprevedenih delov gena *CYP51* pri dveh sesalcih (miš, človek) in eni rastlini (*Arabidopsis thaliana*). Določili smo nukleotidno zaporedje introna 1 in oddaljenega promotorja mišjega gena *Cyp51*. Ugotovili smo, da intron 1 vsebuje potencialne DNA regulatorne elemente (i.e. C/EBP), en del zaporedja pa kodira krajši peptid. V predhodnih raziskavah je bil v promotorski regiji človeškega gena *CYP51* najden začetek gena, ki ustreza mRNA *AL133568* in se prepisuje v obratni smeri kot *CYP51*. S pomočjo internetnih orodij smo preverili ohranjenost tega gena pri drugih sesalcih in njegovo povezanost z genom *CYP51*. Ugotovili smo delno ohranjenost gena *AL133568* pri prašiču, podgani in miši.
Rastlina *A. thaliana* ima dva gena *CYP51* (*CYP51A1* in *CYP51A2*) z še neznano funkcijo. Primerjali smo promotorski regiji obeh rastlinskih genov s promotorskimi regijami sesalcev in skušali določiti regulatorne elemente. Ugotovili smo, da promotor *CYP51A2* gena rastline *A. thaliana* vsebuje potencialne regulatorne elemente, ki se pojavljajo tudi pri sesalcih. Promotor *CYP51A1* pa takšnih regulatornih elementov ne vsebuje.

Analysis of Untranslated Portions of *CYP51* Genes in Eukaryotes

A. Krajnc
Biotechnical Faculty, University of Ljubljana, Slovenia
Supervisor: N. Debeljak
Co-supervisor: D. Rozman
Faculty of Medicine, University of Ljubljana, Slovenia

CYP51 gene encodes lanosterol 14α-demethylase (*CYP51*), a member of the cytocrom P450 superfamily that participates in cholesterol biosynthesis. *CYP51* is one of most conserved *CYP* genes and that is found in eukaryotes and prokaryotes. Our aim was to determine nucleotide sequence and to characterize untranslated regions of *CYP51* genes in two mammals (mouse, human) and one plant (*Arabidopsis thaliana*). We determined nucleotide sequence of intron 1 and of the distant promoter of mouse *Cyp51*. Intron 1 contains potential regulatory elements (*i.e.*C/EBP) and encodes a short peptide. Previous research determined another gene (*AL133568*) residing in the promoter region of the human *CYP51*, being oriented in head-to-head position. We used Internet programs to search whether homologs of AL133568 are present in other mammals, and to examine its connection with *CYP51*. Partially conserved *AL133568* was determined in pig, rat and mouse.
The plant *A. thaliana* contains two *CYP51* genes (*CYP51A1* and *CYP51A2*) with yet unknown function. We compared promoter regions of both plant genes with promoter regions of *CYP51* in mammals and searched potential regulatory elements. Same potential regulatory elements as in mammals were found in promoter region of *CYP51A2* gene but not in *CYP51A1* gene.

Trženjski splet Krkinih zdravilišč na tujih trgih

H. Kralj
Krka Zdravilišča, d. o. o., Slovenija
Mentor: M. Hribar
Krka Zdravilišča, d. o. o., Slovenija
Somentorica: M. Makovec Brenčič
Ekonomska fakulteta, Univerza v Ljubljani, Slovenija

Leto 2004 se hitro približuje, z njim pa tudi mesec maj, ko bo Slovenija postala polnopravna članica Evropske unije. Z vstopom Slovenije v EU bo prišlo do številnih sprememb tako na političnem kot na gospodarskem področju. Da pa slovenska podjetja ne bi »povozil vlak časa« se morajo že sedaj intenzivno pripravljati na bližajoče se nevarnosti in priložnosti, ki jih čakajo. V svojem delu sem analizirala, kako se na EU pripravljajo na področju turizma, predvsem pa v Krkinih Zdraviliščih.
Preden sem analizirala spremembe in posledice vstopa v EU, sem natančno opredelila trženjski splet Krkinih Zdravilišč ter analizirala značilnosti, trende in cilje trenutno najpomembnejših tujih trgov (Nemčija, Avstrija, Italija).
Na splošno so v slovenskem turizmu dokaj optimistično razpoloženi in se ne bojijo vstopa v EU; to velja tudi za Krkina Zdravilišča, saj že sedaj ogromno delajo in sodelujejo z državami EU. Z vstopom v EU se jim bo močno povečal »domači trg«, to je z 2-milijonskega na kar 450-milijonski trg.
Trenutno pa največji problem predstavlja prepoznavnost Slovenije in posledično tudi Krkinih Zdravilišč. Z vstopom se bo takoj močno povečalo število objav o Sloveniji v številnih publikacijah po Evropi. Vendar pa to ne bo dovolj. Turistična podjetja se bodo morala bolj povezati in doseči boljše pozicioniranje Slovenije kot turistične destinacije.
Da bi Krkinim Zdraviliščem uspelo pridobiti še večji delež tujih gostov in biti konkurenčni na močnem evropskem trgu, se bodo morali osredotočiti na oblikovanje številnih potencialnih produktov (tržne niše). Promocijo bodo morali usmeriti predvsem v ponudbo pristnih, naravnih in kulturno avtentičnih turističnih proizvodov, pri tem pa ne pozabiti na visoko stopnjo kakovosti storitev.

Marketing Mix of Krka Zdravilišča on Foreign Markets

H. Kralj
Krka Zdravilišča, d. o. o., Slovenia
Supervisor: M. Hribar
Krka Zdravilišča, d. o. o., Slovenia
Co-supervisor: M. Makovec Brenčič
Faculty of Economics, University of Ljubljana, Slovenia

The year 2004 is approaching fast, along with the month of May when Slovenia will become a full member of the European Union. With the entrance of Slovenia into the EU numerous changes in political as well as in economical fields will occur. To keep Slovenian companies competitive they have to start preparing for the dangers and advantages to come. In my work I have analyzed their preparations in tourism, focusing mainly on Krka Zdravilišča health resorts.
Before I analyzed the changes and consequences of the entrance into the EU I explicitly defined the marketing mix of Krka Zdravilišča and analyzed the characteristics, trends and goals on currently most important foreign markets (Germany, Austria, Italy).
Slovenian tourism sphere is in general rather optimistic and not afraid of the entrance into the EU. This applies also to Krka Zdravilišča, since they already cooperate with the EU members. When joining the EU the home market of 2 millions will expand up to 450 millions.
Currently the greatest problem lies in the recognition of Slovenia and consequently of Krka Zdravilišča. With the entrance into the EU the number of articles about Slovenia will increase in numerous publications around Europe. However, this will not be sufficient. Tourist companies will have to link themselves together and try to achieve better position of Slovenia as a tourist destination.
To achieve even larger share of foreign tourists and to stay competitive on the strong European market Krka Zdravilišča will have to focus on establishing several potential products (market niches). Their promotion will have to be oriented primarily towards providing genuine natural and cultural tourist products, and at the same time not forgetting about the high quality of service.

Suspenzijska kultura rastline *Solanum laciniatum* in biotehnološko pridobivanje steroidnih alkaloidov

T. Kralj
Krka, d. d., Novo mesto, Slovenija
Fakulteta za farmacijo, Univerza v Ljubljani, Slovenija
Mentor: R. Ucman
Krka, d. d., Novo mesto, Slovenija

Rastlinska vrsta *Solanum laciniatum*, ki spada v družino razhudnikovk (*Solanaceae*), vsebuje veliko steroidnih alkaloidov, med katerimi je največ solasodina. Ta se v rastlini nahaja v obliki triglikozida solasonina. Solasodin se v farmacevtski industriji uporablja kot izhodna spojina za pridobivanje steroidnih učinkovin.
Iz listov rastline *Solanum laciniatum* smo pripravili suspenzijsko kulturo ter spremljali prirast biomase in vsebnost steroidnih alkaloidov v različnih rastnih fazah. Koncentracije steriodnih alkaloidov so nihale od 1,6 – 4,0 mg/g suhe mase. Ugotavljali smo tudi vpliv rastnega in produkcijskega gojišča ter elicitorjev (JA, rutin, *Erwinia carotovora* subsp. carotovora Kr72 in *Erwinia chrysanthemi* pv chrysanthemi NCPPB 402) na vsebnost steroidnih alkaloidov. Z elicitacijo (poskus 5 – mrtve in žive bakterije *Erwinia chrysanthemi* pv chrysanthemi NCPPB 402) nam je uspelo signifikantno povečati vsebnost steroidnih alkaloidov.
Steroidne alkaloide smo določevali tudi v listih svežih rastlinah in v *in vitro* pridobljenih rastlinah. Vsebnost steroidnih alkaloidov je bila največja v mladih listih in v *in vitro* pridobljenih rastlinah. Koncentracije so nihale od 5,6 – 7,3 mg/g suhe teže.
Kot analizno metodo za določevanje vsebnosti steroidnih alkaloidov smo uporabili spektrofotometrično analizo.

Suspension Culture of *Solanum laciniatum* and Biotechnological Production of Steroidal Alkaloids

T. Kralj
Krka, d. d., Novo mesto, Slovenia
Faculty of Pharmacy, University of Ljubljana, Slovenia
Supervisor: R. Ucman
Krka, d. d., Novo mesto, Slovenia

Plant Species *Solanum laciniatum* is a member of *Solanaceae* family and contains a large number of steroidal alkaloids, among which solasodine prevails. Solasodine is found in the plant in the form of triglycoside solasonine. Pharmaceutical industry uses solasodine as a raw material for the production of steroidal agents.
We prepared a suspension culture from the leaves of the *Solanum laciniatum* plant and observed the increase of biomass and the content of steroidal alkaloids in different growing phases. The concentration of steroidal alkaloids varied from 1.6 to 4.0 mg/g of dry mass. We also tried to estimate the influence of growth and production medium and elicitors (JA, rutin, *Erwinia carotovora* subsp. carotovora Kr72 and *Erwinia chrysanthemi* pv chrysanthemi NCPPB 402) on the content of steroidal alkaloids. By elicitation (experiment 5 – dead and live bacteria *Erwinia chrysanthemi* pv chrysanthemi NCPPB 402) we managed to significantly increse the content of steroidal alkaloids.
We also tried to determine steroidal alkaloids in the leaves of fresh and *in vitro* prepared plants. Their content was the highest in young leaves and *in vitro* plants. The concentrations varied from 5.6 to 7.3 mg/g of dry mass.
To determine the content of steroidal alkaloids, we used the method of spectrophotometrical analysis.

Pomen posameznih aminokislinskih ostankov pri delovanju 17β-hidroksisteroid-dehidrogenaze iz glive *Cochliobolus lunatus* – priprava in karakterizacija usmerjeno mutiranih proteinov

K. Kristan
Medicinska fakulteta, Univerza v Ljubljani, Slovenija
Mentorica: T. Lanišnik Rižner
Somentor: J. Stojan
Medicinska fakulteta, Univerza v Ljubljani, Slovenija

17β-hidroksisteroid-dehidrogenaza iz glive *Cochliobolus lunatus* (17β-HSDcl) je regiospecifični encim, ki katalizira oksidoredukcije androgenov in estrogenov v prisotnosti koencima NADP(H) in sodi v naddružino kratkoverižnih dehidrogenaz/reduktaz. Z usmerjeno mutiranimi proteini smo proučevali pomen posameznih aminokislinskih ostankov pri delovanju encima.
Rezultati kažejo, da je za encimsko katalizo ključen Tyr167, saj je mutirani protein Tyr167Phe izgubil vso aktivnost. Bližnji His164 pa verjetno predstavlja sterično oviro pri dostopu v aktivni center. Za učinkovito delovanje encima so poleg katalitičnih aminokislin pomembni tudi tisti aminokislinski ostanki, ki stabilizirajo nikotinamidni obroč koencima v aktivnem centru. Mutiranim proteinom Thr202Val, Thr202Ile, Met204Gly in Phe205Gly je encimska aktivnost namreč močno upadla. 17β-HSDcl negativna naboja 2'-fosfatne skupine koencima NADP(H) kompenzira le z eno bazično aminokislino, Arg28. Z uvedbo dodatne bazične aminokisline Arg na mesto Ala50 in Asn51 smo izboljšali encimsko aktivnost. Zamenjava aminokisline Tyr49 z Asp pa je spremenila koencimsko specifičnost iz NADP(H) v NAD(H).

Significance of Individual Amino Acid Residues for Activity of 17β-hydroxysteroid Dehydrogenase from Fungus *Cochliobolus lunatus* – Preparation and Characterization of Mutant Proteins

K. Kristan
Faculty of Medicine, University of Ljubljana, Slovenia
Supervisor: T. Lanišnik Rižner
Co-supervisor: J. Stojan
Faculty of Medicine, University of Ljubljana, Slovenia

17β-hydroxysteroid dehydrogenase from the fungus *Cochliobolus lunatus* (17β-HSDcl) is an NADP(H) dependent enzyme that preferentially catalyses reversible oxidoreductions of androgens and estrogens. It belongs to the short-chain dehydrogenase/reductase superfamily. We prepared mutant proteins and investigated the significance of some amino acid residues for activity of the enzyme.
The results demonstrated the importance of Tyr167 for acid-base catalysis since the mutation Tyr167Phe resulted in a complete loss of activity. His164, which is situated nearby, is probably a steric hindrance toward substrate and/or coenzyme. Amino acid residues, which interact with the nicotinamide ring are essential for an appropriate accommodation of coenzyme in the active site. We elucidated their importance with mutants Thr202Ile, Thr202Val, Met204Gly and Phe205Gly, that showed decreased enzyme activity. 17β-HSDcl compensate the two negative charges of the 2'-phosphate group of the coenzyme NADP(H) by positively charged residue Arg28. Introducing another positively charged residue, arginine, at Ala50 and Asn51 positions, increased the enzyme activity. On the other hand, the substitution of the aspartic acid into the Tyr49 position switched the enzyme's cofactor preference from NADP(H) to NAD(H).

Blood Pressure - Kidney Volume Relationship in Essential Hypertension

M. Laganović
University Hospital Rebro Zagreb, Croatia
Supervisor: Ž. Reiner
University Hospital Rebro Zagreb, Croatia

Objective: our aim was to analyze whether kidney volume determines blood pressure levels, whether lower birth weight contributes future hypertension through lower kidney volume and whether microalbuminuria could be a marker of this pathway.
Patients and methods: we included 103 patients (37+9.1 years) with newly diagnosed essential hypertension (HT) and 92 healthy controls (NT) (35+7.5 years). Blood pressure (BP) was measured using mercury sphygmomanometer and with ambulatory BP monitor. Kidney volume (KV) was determined by ultrasound using an ellipsoid formula and corrected for BSA (CorrKV). Birth weight (BW) was obtained from mothers. Microalbuminuria (MA) was determined in 24h urine sample with nephelometric method
Results: HT had higher weight, BMI, BSA ($p<0.001$), lower BW, higher MA ($p=0.01$), higher sodium in 24 h urine ($p=0.004$) than in NT group. HT had shorter and widther kidneys and larger kidney volume ($p<0.05$). However, when corrected for BSA, the observed corrrelation was not statistically significant ($p=0.13$). We did not observe connection between CorrKV and BP in both groups. HT with lower BW (<3000g) had higher BP ($p=0,03$) than HT with normal BW. We found negative correlation between BW and BP ($r=-0.31$; $p<0.05$ vs. $r=-0.18$; $p>0,05$) in both groups. BW was not correlated with CorrKV except in HT with lower BW ($r=0,64$, $p<0,05$). Both HT and NT with lower BW had longer and thiner kidneys ($p=0,02$). We observed positive correlation between 24h sist. BP and MA ($r=0,46$, $p<0,05$). BW and CorrKV were not correlated with MA. However, correlation was found between 24h sist BP and MA in subgroups with lower BW (<3000g. $r=0,52$, $p<0,05$; <2500g. $r=0,60$, $p<0,05$).
Conclusions: we failed to find association between kidney volume and BP values. BW influences BP values in adult age but it was not mediated through lower kidney volume. Participants with lower BW had longer and thiner kidneys than normal BW group. Microalbuminuria is marker of early renal damage especialy in HT with lower BW. Other mechanisms (i.e. increased BMI, increased salt intake, etc) might be more important for increased BP.

Investigations on the Pharmacological Mechanisms of the Cardioprotective Compound ME10092

E. Liepinsh
Faculty of Medicine, University of Latvia, Riga, Latvia
Supervisor: R. Muceniece
Faculty of Medicine, University of Latvia, Riga, Latvia

The complexity of ischemia and reperfusion-mediated biochemical events in the heart offers multiple possibilities for pharmacological intervention. The guanidine compound ME10092 (1-(3,4-dimethoxy-2-chlorobenzylideneamino)-guanidine) possesses a strong cardioprotective effect to ischemia-reperfusion in rats (2). The present study was undertaken to clarify the biochemical and molecular mechanisms underlying the cardioprotective effect of ME10092. We have evaluated ME10092 for adrenergic activities *in vitro* and *in vivo*, as well as tested the hypothesis that ME10092 interferes with the free radical-related cellular signaling.
We found that ME10092 binds to α_1- and α_2-adrenoreceptors with moderate affinity (K_i values 1-4 μM), and blocks adrenaline elicited contractile responses in isolated guinea pig aortas. In the rat ME10092 decreased the blood pressure and heart rate in a dose dependent manner. Our results also indicate that ME10092 possesses an anti-oxidant profile, i.e., it is inhibitor of xanthine oxidase and NAD(P)H oxidase. *In vitro* ME10092 also inhibited the activities of nitric oxide synthases neuronal NOS and endothelial NOS, but not that of inducible NOS. Furthermore, the antiischemic action of ME10092 was confirmed in a simulated ischemia-reperfusion model using adult rat cardiomyocyte primary culture where compound was shown to protect cells from ischemia related stress stimuli.
Our results show that guanidine derivative ME10092 modulates the action of several enzymatic and receptor systems and thus brings about the cardioprotective and antiischemic effect both *in vivo* and *in vitro*.

1. Wang, Q.D., Pernow, J., Sjoquist, P.O., Ryden, L. Pharmacological possibilities for protection against myocardial reperfusion injury. *Cardiovasc. Res.* **2002,** 55, 25-37.
2. Dambrova, M., Veveris, M., Cirule, H., Pugovichs, O., Post, C., Lundstedt, T., Kalvinsh, I., Skottner, A., Wikberg, J.E. The novel guanidine ME10092 protects the heart during ischemia-reperfusion. *Eur. J. Pharmacology* **2002**, 445,105-113.

Spremljanje stabilnosti trženih izdelkov s poudarkom na roku uporabe

K. Ljubec
Krka, d. d., Novo mesto, Slovenija
Mentorica: M. Medić-Šarić
Farmacevtsko-biokemijska fakulteta, Univerza v Zagrebu, Hrvaška

Pri testiranju stabilnosti trženih farmacevtskih izdelkov postaja zelo pomemben tudi podatek, ali izdelek ohrani lastnosti stabilnosti in učinkovitosti v času roka uporabe.
Namen diplomskega dela je bil preveriti rok uporabe (utilization period, in-use stability). To je časovno obdobje, znotraj katerega se lahko uporabi rekonstitucijski izdelek ali končna zdravilna oblika v odprti multidozni posodi.
Rok uporabe smo spremljali na izdelkih, ki vsebujejo aktivno učinkovino ketokonazol v različnih farmacevtskih oblikah: tabletah, kremi in šamponu. V ta namen smo merili parametre: videz, vsebnost aktivne učinkovine in sorodnih substanc, ter pH vrednost pri kremi in šamponu. Vrednotenje vsebnosti aktivne učinkovine in sorodnih substanc je potekalo z metodo visokotlačne tekočinske kromatografije. Testirali smo po dve seriji vsake od zgoraj navedenih farmacevtskih oblik. Eksperiment smo postavili tako, da smo izbrali serijo na začetku in na koncu roka uporabe. Pogostnost testiranja smo izvedli tako, da smo analizirali serije takoj po odprtju multidozne posode in nato v določenih časovnih intervalih do predvidene porabe zdravila.
Iz dobljenih rezultatov smo preverili stabilnost in učinkovitost znotraj roka uporabe navedenih farmacevtskih oblik, ki vsebujejo aktivno substanco ketokonazol.

Stability Monitoring of Marketed Products with Stress on Utilization Period

K. Ljubec
Krka, d. d., Novo mesto, Slovenia
Supervisor: M. Medić-Šarić
Faculty of Pharmacy and Biochemistry, University of Zagreb, Croatia

Essential data in stability testing of pharmaceutical marketed products is wether they can retain their stability and efficacy throughtout utilization period.
Utilization period is the period of time during which a reconstituted preparation or the finished dosage form in an opened multidose container can be used.
Utilization period of products containing active substance ketokonazol in different dosage forms: tablets, creme, shampoo was tested. Monitored parameters were: organoleptic parameters, active substance assay, degradation product level and pH value of creme and shampoo. Active substance assay and degradation product level were evaluated by HPLC technique. Two batches of each dosage form were tested during experiment. One of the batches was chosen towards the end of its shelf life and the other in the beginning of its shelf life. Test was designed to stimulate the use of the product in practise taking into consideration the filling volume of the multidose container and dosage of medicine described in the product literature.

RP–HPLC and Derivative Spectrophotometry in Multicomponent Drug Analysis

A. Malenović
Faculty of Pharmacy, Belgrade University, Serbia and Montenegro
Supervisors: D. Ivanovic, M. Medenica
Faculty of Pharmacy, Belgrade University, Serbia and Montenegro

INTRODUCTION: The aim of presented master thesis was to develop the optimal conditions of RP–HPLC and derivative spectrophotometric methods which make possible simultaneous determination of: 1. paracetamol, caffeine and propyphenazone in Dalivon® tablets and 2. lidocaine and cetrimonium bromide, in the presence of color corrigent, in Septalen® pellets as well as to validate the developed methods. Validation of an analytical method means comprises an investigation on robustness, selectivity, linearity, precision, limit of detection and limit of quantification. Investigated drugs are an official formulations of Krka, Novo mesto, Slovenia.

EXPERIMENTAL: The chromatographic separations were performed on Hewlett Packard 1100 system which consisted of HP 1100 pump, HP 1100 UV–VIS Detector and HP ChemStation integrator. Separations were performed on a Beckman Ultrasphere ODS 4.6 mm x 15 cm, 5mm particle column at 40°C. Injection volume was 20 mL. For the separation and determination of paracetamol, caffeine and propyphenazone mobile phase consisting of methanol– water (44 : 56 V/V) with flow rate 1 ml/min. Phenobarbital was used as an internal standard. UV detection was performed at 265 nm. Simultaneous determination of lidocaine, cetrimonium bromide and color was performed applying the mobile phase which consisted of acetonitrile– water phase (28 : 72 V/V) with flow rate 1 ml/min. Water phase was prepared by mixing 835 ml of redestillated water and 65 ml of 1.0 $molL^{-1}$ sodium–hydroxide solution. pH was adjusted to 2.0 with 85 % orthophosphoric acid and than the sufficient amount of redestillated water was added up to 1000 ml. Bisacodil was used as an internal standard. UV detection was performed at 208 nm.
Derivative spectrophotometry was performed on Beckman spectrophotometer DU Series 600 with 1 cm quartz cells. Suitable settings were: mode $^2D = d^2A/dl^2$, spectrophotometric wavelength range from 200 nm to 350 nm, scan speed 2400 nm/min, wavelength modulation Dl = 2 nm and smoothing 25 points. For the simultaneous determination of active ingredients in Dalivon® tablets 0.01 mol/L sodium-hydroxide was the solvent and for the Septalen® pellets 0.1 mol/L hydrochloric acid.

RESULTS AND DISCUSSION: The successful analysis of drugs in complex pharmaceutical formulations by the RP–HPLC method relies upon the optimisation of the chromatographic separation. Optimisation of the RP–HPLC method means examination and estimation of factors that have a significant influence on the investigated HPLC system. For defining the optimal chromatographic conditions response surface metodology (RSM) was applied.With the aid of RSM it was possible to anticipate to a certain degree and precisely select optimal experimental conditions. RSM is a graph of system response (separation factor values) as a function of one or more factors, visual means of understanding how certain factors influence the system. The simultaneous influence of mobile phase composition/pH of the mobile phase and mobile phase composition/column temperature on the response of the system was followed.
After establishing the optimal chromatographic conditions, for separation the investigated substances, the proposed method was validated.
Simultaneous determination of drugs in multicomponent formulations could be achieved applying derivative spectrophotometry because it improves resolution which increases with the derivative order. This method is useful for resolving the overlapped spectra and eliminating matrix interference in the assay of a main substance. The derivative spectrophotometric method, for simultaneous determination of paracetamol/caffeine/propyphenazone mixture and lidocaine/cetrimonium bromide mixture in the presence of color corrigent, was developed and than validated. Experimental investigations have showed that resolution and discrimination both increase with derivative order, while the signal to noise ratio (S/N) decrease. For the simultaneous determination, the second–derivative order (^2D) was chosen because it provided a good compromise between resolution and S/N ratio. Paracetamol was determined at 332 nm applying "zero crossing" method. But for the determination of caffeine and propyphenazone it was necessary to correct the peak amplitude and to calculate the factor of correction. The both components were determined according to paracetamol: caffeine at 248 nm and propyphenazone at 257 nm. In the case of lidocaine and cetrimonium bromide the problem was the lack of a strong chromophore in the cetrimonium bromide structure and very low absorbancy even in a higher concentrations. For that reason ^2D was chosen for the simultaneous determination. Lidocaine was determined at 250 nm applying "zero

crossing" method. Cetrimonium bromide determination at 215 nm necessited a correction of the peak amplitude and calculation of correction factor. The signals of the color ingredient were zero at 215 nm and 250 nm.

CONCLUSION: The RP–HPLC and derivative spectrophotometric methods were developed for the determination of: 1. paracetamol, caffeine and propyphenazone mixture, 2. lidocaine and cetrimonium bromide even in the presence of color corrigent that can perform strong interference. The proposed analytical methods were than validated. Selectivity of the proposed methods was proved. Linearity and precission were confirmed calculating an appropriate parameters recquired by the ICH. Robustness of the methods was also investigated and confirmed. The developed derivative spectrophotometric method for the separation of mixture of substances with significantly different polarity is simpler, less expensive and less time–consuming compering to RP–HPLC method. But both methods proved to be reliable and can be proposed for accurate and precise assay, depending on equipment present in the laboratory or knowledge of the technique.

Vpliv podjetja Krka Zdravilišča na preobrazbo kulturne pokrajine na Dolenjskem

J. Novosel
Filozofska fakulteta, Univerza v Ljubljani, Slovenija
Mentor: A. Gosar
Filozofska fakulteta, Univerza v Ljubljani, Slovenija
Somentor: M. Černe
Krka Zdravilišča d. o. o., Novo mesto, Slovenija

Krka Zdravilišča je podjetje, v katero spadajo enote Zdravilišče Dolenjske Toplice, Zdravilišče Šmarješke Toplice, Zdravilišče Strunjan (v nalogi ni obravnavana enota) in Hoteli Otočec. V nalogi so prikazane značilnosti podjetja in posameznih enot, ki se nahajajo na Dolenjskem. Vse tri enote se vse bolj usmerjajo k programom promocije in krepitve zdravja oz. wellness središčem, s čimer sledijo sodobnim tendencam v turizmu. Namen diplomske naloge je primerjati uspešnost enot med seboj, kar sem ovrednotila s pomočjo izbranih kazalcev turističnega prometa (nočitve, povprečna letna zasedenost, povprečna doba bivanja gostov). Najuspešnejša je enota Zdravilišče Šmarješke Toplice, ki kaže najvišjo letno zasedenost med vsemi 15 slovenskimi naravnimi zdraviliščі. Opredeljene so razvojne značilnosti posameznih enot ter vpliv vojnih razmer na turistični promet po letu 1991, katere so zlasti vplivale na poslovanje Hotelov Otočec. Ugotavljala sem monofunkcijsko oz. polifunkcijsko usmerjenost naselij. Turistična oz. zdraviliška dejavnost je v vseh treh naseljih oz. kulturni pokrajini pustila viden pečat, glavni vpliv je na strukturo delovnih mest, gibanje prebivalstva in infrastrukturno opremljenost naselja. Tudi v prihodnje bo glavna gonilna sila pri razvoju obravnavanih naselij.

The Influence of Krka Zdravilišča on the Transformation of Cultural Landscape in Dolenjska

J. Novosel
Faculty of Arts, University of Ljubljana, Slovenia
Supervisor: A. Gosar
Faculty of Arts, University of Ljubljana, Slovenia
Co-supervisor: M. Černe
Krka Zdravilišča d. o. o., Novo mesto, Slovenia

Krka Spas is a company, which unites Thermal Spa Dolenjske Toplice, Šmarješke Toplice, Strunjan (not included in the thesis) and Hotels Otočec. The characteristics of the company and its units in Dolenjska are shown in the study. All three units are focusing more and more on the programs of promotion and strenghtening of health and on developing into wellness centers in order to follow recent trends in tourism. The main goal of this thesis is to compare the success among units, which I have evaluated with the chosen parameters of tourist turnover (overnight stays, average annual occupation, average time of staying). The most successful unit is Šmarješke Toplice with the highest average annual occupation among all 15 Slovenian health resorts. The thesis discusses developing characteristics of each unit and the influence of war time on the tourist turnover after the year 1991, which affected mostly the management and tourist visit of Hotels Otočec. I have determined whether tourist sites are multifunctional or monofunctional tourist resorts. Tourism has exerted considerable impact on all three spas/tourist sites and on cultural landscape, especially the impact on the developement and structure of work posts, the dynamics of population growth and infrastructure. Tourism will be the main bearer of progress also in the future.

Karakterizacija kristalnih oblik spojine K-0805

N. Ograjšek
Fakulteta za farmacijo, Univerza v Ljubljani, Slovenija
Mentorica: B. Kotar-Jordan
Somentor: F. Vrečer
Krka, d. d., Novo mesto, Slovenija

V literaturi nismo našli podatkov o kristalnih oblikah spojine K-0805, komercialno pa je dostopna v obliki dveh hidratov, monohidrata in seskvihidrata. Z različnimi metodami kristalizacije smo pripravili različne kristalne in amorfno obliko spojine.
Za karakterizacijo kristalnih oblik smo uporabili termično analizo (TGA in DSC), IR in ramansko spektroskopijo, rentgensko praškovno difrakcijo in ^{13}C-NMR. Izbranim kristalnim oblikam smo določili tudi pravo gostoto, stični kot, topnost in dinamično sorpcijo vode.
S prekristalizacijo K-0805 iz etil-acetata smo dobili kristalno obliko, ki ima najvišje tališče. Poimenovali smo jo hemihidrat, čeprav je DSC analiza pokazala, da naj bi bil vzorec zmes dveh hidratov. Do dehidratacije hemihidrata, pa tako kot pri monohidratu in seskvihidratu pride šele ob taljenju vzorca. Pri raztapljanju v pufru pH=9 je monohidrat kristaliziral v novo kristalno obliko – poimenovali smo jo dihidrat, ki pa po dehidrataciji pri približno 90°C preide v amorfno obliko. Stični kot glicerola je najmanjši pri monohidratu, kar pomeni, da bi bilo njegovo raztapljanje najhitrejše, saj ima tudi največjo topnost. Pri povišani relativni vlagi (>80%) sta seskvihidrat in dihidrat stabilna, monohidrat in hemihidrat pa prehajata v drugo psevdopolimorfno obliko. Pri sušenju z razprševanjem, liofilizaciji in rotavapiranju smo uspeli pripraviti amorfno obliko, ki ima največjo topnost in tako kot monohidrat v pufru kristalizira v dihidrat. Pri povišani relativni vlagi amorfna oblika ni stabilna, saj prehaja v kristalno obliko.

Characterization of Crystal Structures of the Compound K-0805

N. Ograjšek
Faculty of Pharmacy, University of Ljubljana, Slovenia
Supervisor: B. Kotar-Jordan
Co-supervisor: F. Vrečer
Krka, d. d., Novo mesto, Slovenia

In literature we have found no information about crystal structures of the compound K-0805 which is commercially available as monohydrate and sesquihydrate. We prepared many different crystal and amorphous forms of K-0805 by using different methods of crystallisation.
The crystal forms were studied by thermal analysis (TGA and DSC), IR and Raman spectroscopy, X-ray powder diffractometry and ^{13}C-NMR. Some of the samples were also studied by real density, contact angle, solubility and dynamic water sorption.
By recrystallisation of K-0805 from ethyl acetate we gained the crystal form which has the highest melting point. We named it hemihydrate, although DSC analysis showed that the sample probably exists as the mixture of two hydrates. The dehydration of hemihydrate, monohydrate and sesquihydrate occurs at the melting point of the sample. In the buffer solution pH=9 monohydrate crystallises as dihydrate (a new crystal form) which after dehydration at approximately 90°C becomes amorphous. Monohydrate has the lowest contact angle and the highest solubility which means it has the highest dissolution rate. At the relative humidity over the 80% sesquihydrate and dihydrate are the stable forms, while monohydrate and hemihydrate convert to an another pseudopolymorphic form. By spray drying, liophylization and vacuum evaporation we gained the amorphous form which has the highest solubility and which crystallises to the dihydrate in the buffer solution pH=9. At high relative humidity amorphous form isn't stable, because it converts to crystal form.

Vpliv rastnih in diferenciacijskih dejavnikov v z monociti kondicioniranem mediju na dozorevanje dendritskih celic *in vitro*

T. Ogrič
Zavod RS za transfuzijsko medicino, Ljubljana, Slovenija
Mentor: M. Jeras
Somentorica: U. Repnik
Zavod RS za transfuzijsko medicino, Ljubljana, Slovenija

Dendritske celice (DC) so profesionalne antigen predstavitvene celice, ki igrajo osrednjo vlogo v proženju, nadzoru in usmerjanju imunskega odziva. Z našim delom smo želeli ovrednotiti učinkovitost z monociti kondicioniranega medija (MCM) v postopku priprave zrelih DC *in vitro*.
Iz levkocitnih koncentratov smo izolirali monocite ter iz njih, s citokinoma IL-4 in GM-CSF, pripravili najprej nezrele, nato pa še zrele DC. Slednje smo pridobili tako, da smo nezrelim DC dodali različne dejavnike zorenja: TNF-α, LPS oziroma 10 - 50% MCM in jih gojili od 1 do 4 dni. V tem času smo spremljali spremembe celične morfologije in fenotipskih lastnosti posameznih vrst celic, kakor tudi njihovo sposobnost fagocitiranja delcev dekstrana. V posameznih in združenih vzorcih MCM, ki smo jih pripravili s pomočjo mononuklearnih celic (MNC), izoliranih iz levkocitnih koncentratov različnih oseb, smo izmerili koncentracije citokinov TNF-α, IL-6 in IL-1β ter preučili kinetiko njihovega izločanja. Na osnovi izmerjenih vrednosti ter primerjave dozorevalne učinkovitosti MCM s TNF-α in LPS, smo določili najustreznejši volumski delež MCM v standardnem mediju. Nazadnje smo primerjali tudi vplive združenih MCM, pripravljenih po 18, 21 in 24 urah inkubacije MNC, na dozorevanje DC in ugotovili, da med njimi ni nobenih razlik.
Naši rezultati kažejo, da za dozorevanje DC lahko uporabimo 30 – 50% MCM, pripravljen že po 18 urah inkubacije MNC. Z njim lahko pridobimo povsem zrele DC že po enem dnevu gojenja, kar je primerljivo z LPS, ki velja za enega najučinkovitejših dejavnikov njihovega dozorevanja.

The Influence of Monocyte Conditioned Medium Containing Various Growth and Differentiation Factors on the *in vitro* Maturation of Dendritic Cells

T. Ogrič
Blood Transfusion Centre of Slovenia, Ljubljana, Slovenia
Supervisor: M. Jeras
Co-supervisor: U. Repnik
Blood Transfusion Centre of Slovenia, Ljubljana, Slovenia

Dendritic cells (DC) are professional antigen presenting cells that are essential for initiation of primary immune responses. We decided to evaluate the efficacy of monocyte conditioned medium (MCM) in inducing terminal differentiation of monocyte derived DC *in vitro*.
Therefore we isolated monocytes from buffy coats and incubated them with IL-4 and GM-CSF to generate immature DC. Subsequently we prepared mature DC, using LPS, TNF-α or 10 – 50% MCM. We monitored the changes of DC morphology and phenotype as well as their ability of dextrane phagocytosis. In MCMs, produced during the culture of monocytes on immobilized γ–globulin, we measured concentrations of cytokines TNF-α, IL-6 and IL-1β and evaluated their production kinetics. We also compared the maturation of DC exposed to MCMs, prepared after 18, 21 or 24 hours of MNC incubation and found no difference.
Our results show that we can efficiently generate mature DC by using 30 - 50% MCM, prepared already after 18 hours of MNC incubation. Such DC become fully mature during the first day of culturing, which is comparable to the effect of LPS, considered to be the most potent differentiation factor of DC.

Celostna reološka analiza izbranih poltrdnih farmacevtskih oblik kot model določevanja viskoelastičnih lastnosti in fizikalne stabilnosti

L. Oman
Fakulteta za farmacijo, Univerza v Ljubljani, Slovenija
Mentorica: M. Gašperlin
Fakulteta za farmacijo, Univerza v Ljubljani, Slovenija

A. Sivec
Fakulteta za kemijo in kemijsko tehnologijo, Univerza v Ljubljani, Slovenija
Mentorica: A. Zupančič–Valant
Fakulteta za kemijo in kemijsko tehnologijo, Univerza v Ljubljani, Slovenija

V raziskovalni nalogi smo proučevali in določevali reološke lastnosti poltrdnim dermalnim farmacevtskim oblikam, ki jih Evropska farmakopeja deli na: gele, mazila, kreme, paste, vroče obkladke in zdravilne obliže. Namenjeni so nanašanju na kožo ali površino sluznic, kjer delujejo lokalno ali zgolj zaščitno. Za terapevtski učinek je vanje potrebno vgraditi zdravilno učinkovino, pri zaščitnem delovanju pa zadostuje že prazna podlaga. Poznavanje reološkega obnašanja postaja vedno bolj pomembno, ker s tem pojasnimo mikrostrukturo sistema, lahko izvajamo kontrolo kakovosti izdelkov in tehnoloških procesov ter proučujemo različne vplive kot so: staranje, temperatura, sprememba sestave... Kot modelna sistema za reološko analizo smo izbrali: Karboksimetilcelulozni gel in Neionsko hidrofilno kremo. Kot poltrdna sistema združujeta lastnosti tekočin in trdnih snovi, sta torej viskoelastična in kot taka med najbolj težavnimi za reološko proučevanje. Meritve smo izvajali pri destruktivnih (tokovne krivulje) in pri nedestruktivnih strižnih pogojih (oscilacijski testi in testi lezenja in obnove). Slednji so se izkazali kot primernejši za tovrstne kompleksne sisteme. Pri obeh vzorcih smo določili prevladujoč elastični doprinos k viskoelastičnemu odzivu. Vrednotili smo tudi vpliv dodatka Na-askorbilfosfata v 1% konc. ter povišane temperature (temperatura kože = 32°C) in ugotovili, da ima temperatura večji vpliv kot vgraditev aktivne spojine.
Na osnovi določitve reoloških dinamičnih in statičnih količin v časovnem obdobju 1 meseca smo fizikalno stabilnost praznih podlag ocenili kot ustrezno.

Complete Rheological Analysis of Selected Semisolid Dosage Forms Serving as a Model for Determining Viscoelastic Properties and Storage Time

L. Oman
Faculty of Pharmacy, University of Ljubljana, Slovenia
Supervisor: M. Gašperlin
Faculty of Pharmacy, University of Ljubljana, Slovenia

A. Sivec
Faculty of Chemistry and Chemical Technology, University of Ljubljana, Slovenia
Supervisor: A. Zupančič–Valant
Faculty of Chemistry and Chemical Technology, University of Ljubljana, Slovenia

In our research project we investigated and determined rheological properties of semisolids. According to the European pharmacopoea semisolids are divided into: gels, creams, ointments, pastes, hot compresses and transdermal patches. Semisolids are used for dermal or mucosal application, where they can have a local healing effect or merely a protective one. When a local effect is desired, a certain drug must be incorporated, whereas a protective effect is achieved by the use of a drug-free semisolid only. Knowledge of rheological properties is becoming increasingly important, as this enables us to predict a system's microstructure. Rheological measurments can be used in product quality control and technologycal process control, and are also useful in studies of various influences on different systems

(ageing, temperature, change of composition...). Two semisolids were analysed: Carboxymethylcellulose gel and Nonionic hydrophile cream. Both combine liquid and solid properties, are thus viscoelastic by nature, and are as such one of the most complicated systems for rheological analysis. Flow behaviour was investigated with destructive methods (flow charts) and viscoelastic properties with nondestructive methods (oscillation tests and creep recovery tests). The latter proved to be more appropriate for analyzing such complex samples. In both models, the elastic module (G') prevailed. Specific protocols were created for each sample and influences of: the active compound (Sodium ascorbyl phosphate), elevated temperature (skin temp. = 32^{o}C). Influences of the active compound were more evident. Physical stability of semisolids during one month was also confirmed.

Monoklonska protitelesa proti membranskim proteinom Caco-2 celic

I. Oven
Biotehniška fakulteta, Univerza v Ljubljani, Slovenija
Mentorica: M. Narat
Biotehniška fakulteta, Univerza v Ljubljani, Slovenija

Caco-2 celice so tumorigene humane epitelne celice, ki izvirajo iz adenokarcinoma debelega črevesa in predstavljajo idealen model za *in vitro* študije interakcij v prebavnem traktu. Želeli smo pridobiti monoklonska protitelesa (mAb) proti membranskim proteinom Caco-2 celic. Z ustreznimi protitelesi bi lahko prepoznali in definirali različne membranske proteine, kar bi bilo dobrodošlo orodje pri različnih *in vitro* študijah. Dve miši BALB/c smo imunizirali z ustrezno pripravljenimi Caco-2 celicami. Selekcijo hibridomov smo v prvi fazi izvajali na živih, intaktnih celicah in s tem izbrali le tiste klone, ki so proizvajali protitelesa proti površinskim molekulam. Uspešnost imunizacijskega postopka in kasneje specifičnost protiteles, ki so jih izločali nastali hibridomi, smo preverjali z metodo indirektnega imunoencimskega testa na nitrocelulozni membrani (iDIBA). Na podlagi rezultatov iDIBA testa smo v končni fazi izbrali 26 pozitivnih hibridomov, ki so izločali mAb proti Caco-2 celicam. Da bi izločili tista mAb, ki reagirajo z membranskimi proteini Caco-2 celic, smo le te izolirali in jih z SDS-PAGE elektroforezo ločili po velikosti ter s prenosom po Westernu uspešno prenesli na membrano in izvedli imunoencimsko reakcijo. Izbrali smo 6 klonov, izmed katerih smo za podrobnejšo analizo nadalje izbrali dva. Ker nas je zanimalo, ali so pridobljena mAb specifična samo za proteine Caco-2 celic ali pa gre morda za neke splošne proteine, ki se pojavljajo tudi pri drugih celicah, smo naredili primerjalno imunoencimsko analizo z membranskimi proteini štirih vrst celic, in sicer Caco-2, JEG-3, NSO in MQ-NCSU celic. Pridobili smo mAb, ki so reagirala s proteinom velikosti okrog 50 kDa, ki se nahaja pri Caco-2 celicah, JEG-3 celicah in NSO celicah, in mAb, ki so reagirala s proteinom velikosti okrog 20 kDa, ki se nahaja le pri Caco-2 celicah. Za natančnejšo definicijo zgradbe in funkcije proteinov, ki jih prepoznavajo dobljena mAb, bi morali izvesti še nekatere biokemične, biološke in fiziološke študije.

Monoclonal Antibodies against Caco-2 Cell Membrane Proteins

I. Oven
Biotechnical Faculty, University of Ljubljana, Slovenia
Supervisor: M. Narat
Biotechnical Faculty, University of Ljubljana, Slovenia

The human epithelial cell line Caco-2 was established from a moderately well differentiated colon adenocarcinoma. Because of its enterocytic differentiation this cell line is suitable for *in vitro* studies of interactions in the intestine. We wanted to obtain monoclonal antibodies (mAb) against Caco-2 cell membrane proteins, which would help us to characterize different membrane proteins that are involved in different mechanisms, which would be a useful tool for *in vitro* studies. To produce the mAbs, 2 BALB/c mice were immunized with Caco-2 cells. Immune serum and hybridoma culture supernatants were screened for antibodies against live, intact cells, which helped us to select the clones that produced antibodies against surface molecules. For screening we used indirect dot immunobinding assay (iDIBA). A total of 26 hybridomas secreting mAbs were selected. To determine which mAbs react with the membrane proteins, proteins were isolated and separated by SDS-PAGE electrophoresis and transferred to membrane by Western blot. On the basis of the immunoblotting results 6 clones were selected, from which 2 of them were selected for further analysis. To determine whether obtained mAbs were specific only for Caco-2 cell membrane proteins or they react also with membrane proteins of other cell types, a comparative immunoblot analysis was performed on Caco-2, JEG-3, NSO and MQ-NCSU cell membrane proteins. We obtained mAbs that reacted with a 50 kDa protein of Caco-2, JEG-3 and NSO cells. We also obtained mAbs that reacted with a 20 kDa protein, which appears to be present only on Caco-2 cells. For more detailed definition of structure and function of the proteins that are recognized by the obtained mAbs, further biochemical, biological and physiological studies should be performed.

Kloniranje in izražanje zapisa za tumorski označevalni protein Trop

M. Pavšič
Fakulteta za kemijo in kemijsko tehnologijo, Univerza v Ljubljani, Slovenija
Mentorica: B. Lenarčič
Fakulteta za kemijo in kemijsko tehnologijo, Univerza v Ljubljani, Slovenija

Tumorska označevalna proteina Trop1 in Trop2 sta modularna transmembranska proteina, prisotna na površini skoraj vseh epitelnih celic. Njuno izražanje se znatno poveča ob maligni transformaciji. Njun zunajcelični del je sestavljen iz treh domen, od katerih je ena podobna tiroglobulinski domeni tipa 1A (Tg1A). Podobne domene najdemo tudi v drugih modularnih proteinih. Za nekatere od teh domen je bilo dokazano, da so močni in specifični inhibitorji nekaterih cisteinskih in aspartatnih proteaz.
Z namenom proučiti inhibitorno aktivnost Tg1A domene kot dela proteinov Trop1 in Trop2 smo uspešno izrazili zunajcelični del proteina Trop1 v kvasovki *Pichia pastoris* ter pokazali, da se izraža pretežno v glikoziliranih obliki in da deluje inhibitorno na cisteinsko proteazo papain. Z metodo modeliranja na osnovi homologije smo izračunali tudi atomske koordinate modela Tg1A domene obeh proteinov na podlagi znane kristalne strukture fragmenta invariantne verige p41 v kompleksu s katepsinom L. Modeli kažejo na strukturno podobnost med obema domenama in omenjenim fragmentom v regijah vključenih v interakcije s proteazo, na osnovi česar sklepamo, da bi lahko obe domeni inhibirali določene papainupodobne cisteinske proteaze.
Delo je del večjega projekta s ciljem dobiti vpogled v fiziološko vlogo Tg1A domene proteinov Trop1 in Trop2 na osnovi njihovih biokemijskih in strukturnih značilnosti. Predvidevamo, da je vloga omenjenih domen preprečevanje proteolitske razgradnje proteinov, katerih del so, ali drugih proteinov zaradi delovanja specifičnih zunajceličnih proteaz, katerih koncentracija se znatno zviša v okolici metastazirajočih novotvorb.

Cloning and Expression of Tumor Marker Protein Trop

M. Pavšič
Faculty of Chemistry and Chemical Technology, University of Ljubljana, Slovenia
Supervisor: B. Lenarčič
Faculty of Chemistry and Chemical Technology, University of Ljubljana, Slovenia

Tumor marker proteins Trop1 and Trop2 are modular transmembrane proteins and are present on the surface of majority of epithelial cells. Their expression is markedly increased in the case of malignant transformation. Their extracellular part is composed of three domains, one being the thyroglobulin type 1A domain (Tg1A). Similar domains can also be found in other modular proteins. Some of those domains are known as strong and specific inhibitors of various cysteine and aspartic proteases.
In order to study the inhibitory activity of Tg1A doman of Trop1 and Trop2 we successfully cloned and expressed extracellular part of Trop1 in methylotrophic yeast *Pichia pastoris* and we have showed that it is predominantly expressed in glycosylated form and that it inhibits cysteine protease papain. Using homology modeling we also computed models of Tg1A domains of Trop1 and Trop2 on the basis of the known crystal structure of p41 invariant chain fragment in complex with cathepsin L. The models show that the regions which govern the interactions with the protease are structurally similar to the respective regions of p41 fragment. Therefore we conclude that it is likely that the Tg1A domain of Trop1 and Trop2 may also inhibit specific members of the papain-family of cysteine proteases.
Our work is part of a larger project which aims to provide insight into physiological role of Tg1A domain of Trop1 and Trop2 on the basis of biochemical and structural information. It is tempting to speculate that they confer resistance to proteolytic cleavage of their cognate or other proteins by specific extracellular proteases associated with malignant transformation.

Uporaba heptapeptidne predstavitvene bakteriofagne knjižnice pri preučevanju medproteinskih interakcij

M. Ploštajner
Fakulteta za farmacijo, Univerza v Ljubljani, Slovenija
Mentor: B. Doljak
Fakulteta za farmacijo, Univerza v Ljubljani, Slovenija

Bakteriofagno predstavitveno knjižnico sestavlja množica bakteriofagov, ki imajo na svoji površini izražene naključne peptide. Oligonukleotid, ki kodira peptid, je namreč vstavljen v gen, ki nosi zapis za plaščni protein bakteriofaga. Z uporabo bakteriofagnih predstavitvenih knjižnic lahko na hiter in preprost način iz množice mikroorganizmov selekcioniramo takšne, ki bodo izkazovali afiniteto do tarčne molekule. Takšni peptidi bi lahko predstavljali spojine vodnice pri razvoju novih učinkovin.
V pričujočem delu smo pri delu uporabili Ph.D.-C7C bakteriofagno heptapeptidno predstavitveno knjižnico. Iz množice naključnih heptapeptidov smo selekcionirali takšne, ki so izkazovali afiniteto do vezave na tarčno molekulo streptavidin in trombin. Streptavidin je tetramerni protein, katerega poglavitna lastnost je izredno močna nekovalentna vezava 4 molekul biotina in predstavlja modelno substanco pri študiju medproteinskih interakcij.
Selekcijski postopek smo pričeli z inkubacijo bakteriofagne predstavitvene knjižnice nad tarčno molekulo, ki smo jo predhodno imobilizirali na površino mikrotitrske ploščice. Fage, ki se niso vezali na tarčno molekulo, smo odstranili z izpiranjem, vezane fage pa smo eluirali s prekinitvijo interakcij, ki so se vzpostavile med fagi in imobilizirano tarčno molekulo. Dobljeni eluat smo pomnožili ter selekcijski postopek ponovili 4-krat. Tako smo v eluatu ohranili le fage z največjo afiniteto do tarčne molekule. 14 selekcioniranim bakteriofagnim klonom smo določili nukleotidno zaporedje, ki nosi zapis za vstavljen heptapeptid in ga nato prevedli v aminokislinsko zaporedje. Znotraj teh smo poskušali poiskati ponavljajoče aminokislinske motive, ključne za vezavo na tarčno molekulo.

Study of Protein-Protein Interactions Using Heptapeptide Phage Display Library

M. Ploštajner
Faculty of Pharmacy, University of Ljubljana, Slovenia
Supervisor: B. Doljak
Faculty of Pharmacy, University of Ljubljana, Slovenia

Phage display library consists of pool of phage expressing different peptides on their surface. The peptide is coded by specific sequence of DNA which is fused to certain bacteriofage coat protein. Phage display peptide library can be used to isolate peptides that bind with high specificity and affinity to any target protein. These binding peptides can be used as lead molecules in drug design.
In this study we used a cyclic heptapeptide phage display library Ph.D.-C7C to select phages that bind to target molecule streptavidin and trombin. Streptavidin, tetrameric protein, binds the small molecule biotin with high affinity and is often used as a model for study protein-protein interactions in biotechnology.
A selection procedure called biopanning has been carried out by incubating the library with a target molecule that has been immobilized on the surface of wells of microtiter plate. Unbound phage we have washed away and the specifically bound phage we have eluted by disrupting the binding interactions between the phage and target. The eluted phage have been then amplified and the process has been repeated for four rounds until only phage displaying specific, tight-binding peptides remain. We have characterized 14 selected individual clones by DNA sequencing and translating into heptapeptide. Within heptapeptides we have determined the motives of three or four amino acides that were repeated more than twice and that gives the best fit to the binding site of the target molecule.

Molekularnogenetska analiza genov *K-ras*, *Rassf1A* in *p53* pri bolnikih z rakom glave in vratu, pljuč in širokega črevesa ter napovedni pomen mutacij teh genov

D. Podpečnik
Medicinska fakulteta, Univerza v Ljubljani, Slovenija
Mentor: D. Glavač
Medicinska fakulteta, Univerza v Ljubljani, Slovenija

Rak je genetsko heterogena bolezen, katere genetske osnove še niso dokončno raziskane. Osrednjo vlogo pri nastanku raka imajo mutacije tumorsko zaviralnih genov, kot sta *Rassf1A* in *p53*, in onkogenov, kot je *K-ras*. V naši raziskavi smo določili pojavnost mutacij in polimorfizmov na zaporedjih DNA gena *K-ras* eksonov 1 in 2, gena *Rassf1A* eksonov 1-6 in gena p53 eksonov 5-8 pri raku širokega črevesa (546 bolnikov), raku glave in vratu (160 bolnikov) ter pljučnemu raku (97 bolnikov), ki so med najpogostejšimi vrstami raka v Sloveniji. Spremembe v preiskovanih odsekih DNA smo najprej zaznali z analizo konformacij, nato pa jih določili s sekveniranjem. Pri bolnikih z rakom širokega čevesa smo odkrili 35-odstotni delež mutacij gena *K-ras* z 8 novimi mutacijami in 12-odstotni delež mutacij gena *p53* z 10 novimi mutacijami. Pri bolnikih z rakom glave in vratu smo odkrili 11-odstotni delež mutacij gena *Rassf1A* in 32-odstotni delež mutacij gena *p53* z 21 novimi mutacijami. Pri bolnikih s pljučnim rakom smo odkrili 13-odstotni delež mutacij gena *K-ras* z eno novo mutacijo, 22-odstotni delež mutacij gena *Rassf1A* prav tako z eno novo mutacijo in 32-odstotni delež mutacij gena *p53* z 11 novimi mutacijami. Na novo odkrite mutacije pri proučevanih vrstah rakavih obolenj lahko pomenijo značilnost slovenskih bolnikov z rakom. Ocenili smo tudi napovedni pomen mutacij genov *K-ras*, *Rassf1A* in *p53*. Pri bolnikih z rakom širokega črevesa imajo mutacije gena *p53* na splošno dober, nekateri posamezni tipi mutacij pa slab vpliv na preživetje. Pri bolnikih z rakom širokega črevesa brez opravljene molekularnogenetske analize za gen *p53* in pri bolnikih s pljučnim rakom imajo slab vpliv na preživetje nekatere mutacije gena *K-ras*. Pri bolnikih z rakom glave in vratu imajo slab vpliv na preživetje nekatere mutacije gena *p53*. Mutacije genov *K-ras*, *Rassf1A* in *p53* so se izkazale za pomembne molekularnogenetske označevalce, ki lahko pripomorejo k učinkovitejšemu odkrivanju posameznih vrst raka in s tem k zdravljenju bolnikov. Pomembne pa so tudi za napoved nadaljnjega poteka bolezni.

Molecular Genetic Analysis of the *K-ras*, *Rassf1A* and *p53* Genes in Patients of the Head and Neck, Lung and Colon Cancer and Prognostic Value of the Mutations of this Genes

D. Podpečnik
Faculty of Medicine, University of Ljubljana, Slovenia
Supervisor: D. Glavač
Faculty of Medicine, University of Ljubljana, Slovenia

Cancer is genetically heterogeneus disorder. The molecular mechanisms of cancer are still not fully understood. Mutations of tumor suppressor genes such as *Rassf1A* and *p53* and oncogenes such as the *K-ras* play a central role in tumorigenesis. We estimated the presence of *K-ras* (exons 1 and 2), *Rassf1A* (exons 1-6) and *p53* (exons 5-8) mutations and polymorphisms in colon (546 patients), head and neck (160 patients) and lung cancer (97 patients) that are the most common types of cancer in Slovenia. Differences in DNA sequences were analysed by conformaton analysis followed by sequencing. K-ras mutations were observed in 35% of patients and p53 mutations in 12% of patients with colon cancer. Of these, 8 new *K-ras* mutations and 10 new *p53* mutations were identified. 11% of patients with head and neck cancer had *Rassf1A* mutations and 32% had *p53* mutations. 21 new *p53* mutations were identified. In lung cancer 13% of patients had *K-ras* mutations (1 new mutation), 22% of patients had *Rassf1A* mutations (1 new mutation) and 32% of patients had *p53* mutations (11 new mutations). New mutations identified in studied cancers may reveal attributes of the Slovenian cancer patients. Additionally, the prognostic significance of *K-ras*, *Rassf1A* and *p53* mutations was evaluated. *p53* mutations in general

have a positive effect on the survival of patients with colon cancer whereas some types of mutations have a negative effect on survival. Some *K-ras* mutations have negative effect on the survival of patients with colon cancer not analysed for *p53* gene mutations and of patients with lung cancer. In patients with head and neck cancer some *p53* mutations have negative effect on survival. Mutations of the *K-ras*, *Rassf1A* and *p53* genes appear to be important molecular genetic markers of cancer. By using this markers we could increase the efficiency of detection of individual cancer types and therapeutic intervention. Mutations are important as prognostic factor for cancer diseases as well.

Validacija potenciometrične metode za določanje vsebnosti benzalkonijevega klorida v Septoletah®

F. Pogorelc
Fakulteta za kemijo in kemijsko tehnologijo, Univerza v Ljubljani, Slovenija
Mentorica: K. Kogej
Fakulteta za kemijo in kemijsko tehnologijo, Univerza v Ljubljani, Slovenija
Somentor: A. Gartner
Krka, d. d., Novo mesto, Slovenija

Delo obsega validacijo potenciometrične določitve vsebnosti benzalkonijevega klorida v Septoletah® s surfaktantno ionoselektivno elektrodo. Dobljene rezultate smo primerjali z rezultati druge do sedaj uporabljane metode za določanje površinsko aktivnih snovi, to je tako imenovana dvofazna titracija. Vsi parametri validacije so bili v zahtevanih mejah. To dokazuje, da je potenciometrična metoda primerna za določanje benzalkonijevega klorida v pastilah Septolete®.
Primerjava rezultatov potenciometrične metode z rezultati dvofazne titracije je dodatno potrdila točnost in primernost metode, saj se dobljeni rezultati razlikujejo v okviru eksperimentalne napake.
Z uporabo potenciometrične titracije se izognemo uporabi kloroforma, kar omogoča varnejše delo in manjše obremenjevanje okolja.

Validation of the Potentiometric Method for the Determination of the Content of Benzalkonium Chloride in Septolete®

F. Pogorelc
Faculty of Chemistry and Chemical Technology, University of Ljubljana, Slovenia
Supervisor: K. Kogej
Faculty of Chemistry and Chemical Technology, University of Ljubljana, Slovenia
Co-supervisor: A. Gartner
Krka, d. d., Novo mesto, Slovenia

The work includes validation of the potentiometric determination of the content of benzalkonium chloride in Septolete® lozenges with the use of ion–selective surfactant electrode. These results were compared with the ones obtained with another method for the determination of surface–active agents, this is the so called two–phase titration. All validation parameters were within the requested limits. This proves that potentiometric method is suitable for the determination of benzalkonium chloride in lozenges Septolete®. Comparison of results obtained by potentiometric method with results of two–phase titration is ion additional conformation that the new method is accurate and suitable for this analysis, i.e., the results of both methods agree within the experimental error. By applying the potentiometric method, the use of chloroform can be omitted. This leads to safer laboratory work and less pollution for the environment.

Vpliv tumor nekrotizirajočega dejavnika-α na izločanje interlevkina-6 iz človeške skeletne mišice v kulturi

O. Prelovšek
Medicinska fakulteta, Univerza v Ljubljani, Slovenija
Mentor: Z. Grubič
Somentor: T. Marš
Medicinska fakulteta, Univerza v Ljubljani, Slovenija

Tumor nekrotizirajoči dejavnik-α, oziroma faktor-α (TNF-α) je pleiotropni citokin, ki kot del kompleksnega citokinskega sistema uravnava sintezo in ekspresijo drugih citokinov in njihovih receptorjev prek aktivacije citokinskih kaskad. V zadnjem času so odkrili nove, neposredne vplive TNF-α na skeletno mišico v procesih, ki povzročajo ali spremljajo razna kritična klinična stanja kot so mišična usahlost, sepsa in kaheksija.
Namen naše naloge je bil proučiti učinek TNF-α na izločanje interlevkina-6 (IL-6) iz človeške mišice v kulturi, in sicer na stopnjah pred in po fuziji mioblastov v mišične cevčice. Kulturam, pripravljenim iz satelitskih celic, ki smo jih izolirali iz koščkov skeletnih mišic, ki se po doktrini odstranijo in zavržejo pri nekaterih ortopedskih operacijah, smo dajali TNF-α v treh različnih koncentracijah. Ugotovili smo, da TNF-α koncentracijsko odvisno pospešuje izplavljanje IL-6. Količine izplavljenega IL-6 so bile v primeru mišičnih cevčic večje kot v primeru mioblastov, kar kaže na odvisnost učinka TNF-α od stopnje mišične diferenciacije. Prisotnost receptorjev za TNF-α smo dokazali s tehniko Western blot. Morebitne povečane apoptoze, ki smo jo ugotavljali s fragmentacijo DNA, nismo ugotovili. Naši rezultati podpirajo aktivno vlogo skeletne mišice pri citokinskem signaliziranju v kritičnih kliničnih stanjih.

The Influence of Tumor Necrosis Factor-α on the Secretion of Interleukin-6 from the Cultured Human Skeletal Muscle

O. Prelovšek
Faculty of Medicine, University of Ljubljana, Slovenia
Supervisor: Z. Grubič
Co-supervisor: T. Marš
Faculty of Medicine, University of Ljubljana, Slovenia

Tumor necrosis factor-α (TNF-α) is a pleiotrophic cytokine which, as a part of complex cytokine system, controls the synthesis and expression of other cytokines and their receptors through the activation of cytokine cascades. The aim of our study was to test the effects of TNF-α on the secretion of interleukin- 6 (IL-6) from cultured human muscle. We studied these effects before and after the fusion of myoblasts into multinucleated myotubes. Cultures prepared from satellite cells isolated from the muscle pieces routinely discarded at some orthopaedic operations were treated with TNF-α at three different concentrations. We found that TNF-α increases the IL-6 secretion in a concentration dependent manner. Quantitatively, secretion from myotubes exceeded the secretion from myoblasts indicating that secretion is differentiation stage-dependent. The presence of TNF-α receptor was confirmed by Western blot. Apoptosis, followed by DNA fragmentation, remained unchanged. Our results support the concept that skeletal muscles actively participate in cytokine signalling in critical clinical conditions.

Študij vpliva velikosti delcev modelne učinkovine KD-9102 na proces vlažne granulacije v hitrem mešalniku

M. Pucelj
Fakulteta za farmacijo, Univerza v Ljubljani, Slovenija
Mentor: O. Planinšek
Fakulteta za farmacijo, Univerza v Ljubljani, Slovenija
Somentor: F. Vrečer
Krka, d. d., Novo mesto, Slovenija

V proizvodnji farmacevtskih izdelkov je za kakovost končnega izdelka izredno pomembna pravilna izbira procesnih in formulacijskih spremenljivk.
V nalogi smo različno mletim delcem modelne učinkovine KD-9102 določili tehnološko pomembne lastnosti in ugotovili, da mletje učinkovine vpliva na velikost delcev, močenje, površinsko energijo in specifično površino delcev, vse to pa vpliva na hitrost raztapljanja. S popolnim 3-faktorskim 2-nivojskim eksperimentalnim načrtom smo nato proučevali vpliv neodvisnih (velikost delcev modelne učinkovine KD-9102, količina prebitne vode v tekočini za aglomeriranje in čas gnetenja) spremenljivk pri procesu granulacije v hitrem mešalniku na lastnosti granulata in iz njega izdelanih tablet. Iz rezultatov analiz smo ugotovili, da je velikost granul večja v primeru daljšega gnetenja. Pri proučevanju raztapljanja granulata smo dokazali, da koncentracija površinsko aktivne snovi v mediju za raztapljanje vpliva na hitrost raztapljanja učinkovine KD-9102. Ugotovili smo, da na hitrost raztapljanja v mediju z nižjo koncentracijo površinsko aktivne snovi najbolj vplivajo velikost delcev učinkovine KD-9102 in čas gnetenja, v primeru višje koncentracije površinsko aktivne snovi pa čas gnetenja in količina prebitne vode v tekočini za aglomeriranje. Proučevali smo tudi kinetiko raztapljanja tablet. Rezultati so pokazali, da se učinkovina iz tablet raztaplja hitreje kot iz granulata ter da na hitrost raztapljanja tu najbolj vplivata čas gnetenja in velikost delcev učinkovine KD-9102.

Study of Influence of Particle Size of Model Drug KD-9102 on Wet Granulation in High-Shear Mixer

M. Pucelj
Faculty of Pharmacy, University of Ljubljana, Slovenia
Supervisior: O. Planinšek
Faculty of Pharmacy, University of Ljubljana, Slovenia
Co-supervisor: F. Vrečer
Krka, d. d., Novo mesto, Slovenia

The appropriate choice of process and formulation variables has a great influence on the quality of final product in the production of pharmaceutical drugs. We defined technological important properties of different milled drug labelled as KD-9102 and found that milling influences particle size, wetting, surface energy and specific surface area which are in correlation with the dissolution rate of drug KD-9102. In the next step we studied the influence of independent variables of wet agglomeration in high-shear mixer (particle size of model drug KD-9102, volume of water in agglomeration liquid and massing time) on the quality of granules and tablets by 3-factorial 2-level experimental design. We found out that size of granules was larger in the case of longer massing time. It was proved that concentration of surface-active substance in dissolution liquid influenced dissolution rate of drug KD-9102 from granules. It was found out that dissolution of the drug in the medium with lower surface-active agent concentration is dependent on particle size and massing time. At higher surface-active agent concentration massing time and volume of water in agglomeration liquid have the highest influence on dissolution. We also studied tablet dissolution kinetics. We found out that drug dissolved faster from tablets than from granules and that massing time and particle size of drug KD-9102 had the most influence on dissolution rate of drug KD-9102 from tablets.

Oxidative Modification of Low Density Lipoproteins in Coronary Heart Disease and its Pharmacological Correction

Y. I. Ragino
Siberian Department of RAMS, Novosibirsk, Russia
Supervisor: I. Nikitin
Siberian Department of RAMS, Novosibirsk, Russia

Today one of the leading theories in the concept of atherosclerotic pathogenesis underlines the vital role of low-density lipoprotein oxidation (LDL). Oxidative – antioxidative potential of LDL was evaluated by biochemical methods in patients with coronary heart disease (CHD) and hypercholesterolemia (HCS) and in patients with arterial hypertension (AH). In the evaluation process the original research methods were used (2 patents from the RF).
The study included 380 patients aged 37 – 60 years. 231 of them had CHD (according to coronary angiography or the history of myocardial infarction) with HCS and 54 patients had arterial hypertension. 95 patients were included in the control group. The investigations included the following: indexes of blood lipid profile, concentration of liposoluble antioxidants of α-tocopherol and retinol in LDL, indexes of the initial level of lipid peroxide oxidation (LPO) in LDL and LDL resistance to oxidation *in vitro*. Additionally, the influence of HMG-CoA reductase inhibitors (lovastatin, simvastatin, atorvastatin and cerivastatin) and of ACE inhibitors (enalapril) on chosen indexes was also estimated.
In comparison with the control group, the patients with CHD and HCS showed a decrease in CH-HDL level by 1.7 times, an increase in TG level by 1.4 times, a significant increase in the initial level of LPO in LDL ($p<0.01$), a low LDL resistance to oxidation ($p<0.05$) and a decrease in α-tocopherol concentration in LDL by 1.3 times, and in retinol concentration by 1.4 times. In patients with AH some similar, but less significant changes of indexes were noticed.
According to our data, the evaluated statins exerted different influences on LDL oxidative modification. In patients suffering from CHD and HCS who received Vasilip, the LDL resistance to oxidation increased ($p<0.05$), but after administration of atorvastatin – the resistance slightly decreased ($p<0.05$). The administration of Holetar (lovastatin) and Lipobay (cerivastatin) did not cause any significant changes to that index. A decrease in LPO level in LDL and an increase of their resistance to oxidation were observed after the patients, suffering from arterial hypertension, received enalapril.
It can be concluded that the potential atherogenic oxidative changes in LDL are obvious in CHD and HCS, and in AH: high level of LPO in LDL, decreased LDL resistance to oxidation and low level of antioxidants in LDL. The study revealed favorable antiatherogenic effects of Vasilip (simvastatin produced by Krka) and enalapril on the LDL oxidative modification.

Vpliv sertralina in amitriptilina na kinetiko eksogenega histamina v krvi mačke v *in vitro* poskusih

S. Rajtar
Medicinska fakulteta, Univerza v Ljubljani, Slovenija
Mentorica: T. Irman Florjanc
Medicinska fakulteta, Univerza v Ljubljani, Slovenija

Raziskave na področju farmakokinetike histamina so pokazale, da antidepresivi lahko spremenijo farmakokinetiko eksogenega histamina pri mački in podgani. Da bi ugotovili, v kolikšni meri pri tej spremembi sodelujejo krvne celice, smo naredili *in vitro* poskuse na mačji krvi. Po odvzemu krvi smo krvnim celicam ali celokupni krvi dodajali antidepresiva amitriptilin ali sertralin in eksogeni histamin. Po inkubaciji smo vzorce čistili z ionsko-izmenjevalno kromatografijo na kolonah in z ekstrakcijo z organskimi topili. Koncentracijo histamina v vzorcih smo določali sprektrofluorometrično. Rezultati so pokazali, da tako amitriptilin kot sertralin *in vitro* povečata privzem eksogenega histamina v krvne celice mačke, amitriptilin za 25%, sertralin pa za 42%. Meritve koncentracij histamina v plazmi bogati s trombociti in v plazmi brez trombocitov, so pokazale, da trombociti sodelujejo pri povečanem privzemu histamina v celice, zaradi delujočega antidepresiva. Ugotovili smo tudi, da se je koncentracija privzetega histamina med inkubacijo krvnih celic znižala, vendar je bilo znižanje manjše v vzorcih s predhodno dodanim antidepresivom. Sklepamo, da amitriptilin in sertralin vplivata na privzem eksogenega histamina v krvne celice mačke in na biotransformacijo histamina.

The Influence of Sertraline and Amitriptyline *in vitro* on the Kinetics of Exogenous Histamine in Feline Blood

S. Rajtar
Faculty of Medicine, University of Ljubljana, Slovenia
Supervisor: T. Irman Florjanc
Faculty of Medicine, University of Ljubljana, Slovenia

The researches in the field of histamine pharmacokinetics have shown that antidepressants can modify the pharmacokinetics of exogenous histamine in cats and rats. In order to examine whether and to what extend blood cells participate in the modification of exogenous histamine kinetics we performed *in vitro* tests on cat blood. After taking the blood samples, an antidepressant, amitriptyline or sertraline, followed by histamine was added to the blood cells or to the whole blood. After the incubation the samples were first cleaned-up with column liquid chromatography and extraction with organic solvents and then the concentration of histamine in the samples was determined spectroflourometrically. The results indicate that both, amitriptyline and sertraline, in vitro increase uptake of exogenous histamine in feline blood cells, amitriptyline by 25% and sertraline by 42%. Measurements of histamine in platelet rich plasma and in plasma without platelets indicate that platelets play an important role in increased histamine uptake. The experiments showed also that during incubation period concentrations of exogenous histamine in the blood cells was decreasing; moreover, decreasing was lower in the cells pretreated with an antidepressant. It can be suggested that amitriptyline and sertraline *in vitro* increase uptake of exogenous histamine in feline blood cells and inhibit the metabolism of exogenous histamine in these cells.

Fibrinolitično, antiagregacijsko in antikoagulacijsko zdravljenje z medicinskega in družbenega vidika

P. Rudolf
Fakulteta za farmacijo, Univerza v Ljubljani, Slovenija
Mentor: J. Drinovec
Krka, d. d., Novo mesto, Slovenija

Antitrombotične učinkovine delimo na fibrinolitike, antiagregacijske in antikoagulacijske učinkovine. Omenjene skupine zdravil smo želeli predstaviti s kliničnega, epidemiološkega, farmakoepidemiološkega in farmakoekonomskega vidika, predvsem v Sloveniji, pa tudi v primerjavi z razvitim svetom.
Poraba antitrombotičnih zdravil v Sloveniji je v vseh podskupinah zdravil manjša v primerjavi z Evropo kot celoto, Nemčijo in skandinavskimi državami, razen nefrakcioniranega heparina, katerega poraba je v Sloveniji podobna kot v razvitih državah. Izstopa pa bistveno večja poraba klopidogrela v Sloveniji kot poraba v omenjenih državah, kar močno poviša stroške za zdravila.
Skupni enoletni stroški fibrinolitičnega, antiagregacijskega in antikoagulacijskega zdravljenja vseh bolnikov v Sloveniji, ki bi to zdravljenje potrebovali znašajo 33.826 milijonov SIT. Od tega predstavlja največji delež bolniški stalež, in sicer 29%, sledijo stroški za zdravila (20%), potni stroški (19%), stroški hospitalizacije (16%), stroški za specialista (12%) ter stroški za splošnega zdravnika (4%). To bi pomenilo 10,02% letnih sredstev za zdravstvo in 0,71% bruto družbenega prihodka.
Fibrinolitiki, antiagregacijske in antikoagulacijske učinkovine pomembno zmanjšujejo obolevnost in smrtnost pri kardiovaskularnih boleznih, omogočajo kronično hemodializno zdravljenje in preprečujejo trombembolične zaplete pri obsežnejših operacijah.

Medical and Social Aspects of Fibrinolytic, Antiplatelet and Anticoagulant Therapy

P. Rudolf
Faculty of Pharmacy, University of Ljubljana, Slovenia
Supervisor: J. Drinovec
Krka, d. d., Novo mesto, Slovenia

Antithrombotic agents include fibrinolytic, antiplatelet and anticoagulant agents. The aim of this work was to present these agents from the clinical, epidemiological, pharmaco-epidemiological and pharmaco-economic aspects, mainly in Slovenia and also in comparison to the developed countries.
In Slovenia, the consumption of antithrombotic agents of all types is lower than in Europe as a whole, in Germany and in Scandinavian countries, except for the standard heparin; the consumption of the latter in Slovenia is comparable to that in developed countries. On the other hand, the consumption of clopidogrel in Slovenia is considerably higher than in the above countries, which contributes to the high national expenses for medicines.
The total annual cost of fibrinolytic, antiplatelet and anticoagulant treatment for all patients in Slovenia that should receive such treatment is 33.826 million tollars. The highest percentage of this amount represents the cost of paid sick leave (29%), followed by the cost of medicines (20%), travelling expenses (19%), hospitalization cost (16%), costs for medical specialists (12%), and costs for general practitioners (4%). This would represent 10.02% of the money intended annually for public health expenses and 0.71% of the gross domestic product (GDP).
Fibrinolytic, antiplatelet and anticoagulant agents significantly decrease morbidity and mortality in cardiovascular diseases, make possible chronic hemodialysis and help prevent thromboembolism in major surgery.

Volumske lastnosti derivatov 1,4-dihidropiridina v različnih organskih topilih

L. Rus
Fakulteta za farmacijo, Univerza v Ljubljani, Slovenija
Mentorica: M. Bešter Rogač
Fakulteta za kemijo in kemijsko tehnologijo, Univerza v Ljubljani, Slovenija

Poleg fenilalkilaminov in benzotiazepinov predstavljajo derivati dihidropiridina pomemebno skupino kalcijevih antagonistov, ki se uporabljajo v terapiji hipertenzije. Vse skupine imajo podobne farmakokinetične lastnosti: izrazit prvi prehod po peroralni aplikaciji, velik očistek in vezavo na plazemske proteine. Derivati dihidropiridina učinkujejo neposredno na kalcijeve kanale v celični membrani in blokirajo pretok kalcijevih ionov iz ekstracelularnega medija v celično plazmo. Pri tem igra pomembno vlogo solvatacija funkcionalnih skupin, kot tudi njihove strukturne, dinamične in konformacijske lastnosti, ki jih lahko raziskujemo z določanjem volumskih lastnosti raztopin. Za predmet naše študije smo si izbrali štiri derivate 1,4-dihidropiridina: amlodipin v obliki besilata, nimodipin, nitrendipin in nifedipin. Ker so v vodi zelo slabo topni, smo raziskavo izvedli v acetonu, etanolu, DMSO in diklorometanu. Izmerili smo gostote raztopin amlodipin besilata v etanolu in DMSO ter nimodipina, nitrendipina in nifedipina v vseh topilih pri 25°C v koncentracijskem območju med 0.005 in 0.1 mol dm^{-3}. Iz eksperimentalnih rezultatov smo izračunali navidezne molske volumne in ocenili navidezne molske volumne v neskončno razredčenih raztopinah, kjer lahko predpostavimo samo interakcije topljenec-topilo. Parcialne molske volumne smo določili tudi s pomočjo programa ACD/ChemSketch in Traubejevega koncepta aditivnosti, ki sicer veljajo za vodne raztopine pri 25°C.
Lastnosti topil smo opisali s Hansenovimi parametri in ugotovili, da na spremembo volumna najbolj vplivata parameter vodikove vezi in parameter polarnosti.

Volumetric Properties of 1,4-Dihydropyridin Derivates in Different Organic Solvents

L. Rus
Faculty of Pharmacy, University of Ljubljana, Slovenia
Supervisor: M. Bešter Rogač
Faculty of Chemistry and Chemical Technology, University of Ljubljana, Slovenia

Beside phenylalkylamines and benzotiazepines derivates of dihydropyridine constitute an important class of calcium antagonists widely used in antihypertensive treatment. All groups have very similar pharmacokinetics properties: a distinctive first transition at per oral application, extensive clearance and strong binding on plasmas proteins. Dihydropyridine derivates acting directly on the calcium cannels in the cell membrane and block the flow of calcium ions from the ekstracellular field into the cell plasma. As long-acting calcium channel blockers they act remarkably well in preventing cardiovascular complications at hypertensive patients. The partial molar volume of the substances is a macroscopic observable quantity which is sensitive to the solvation properties of functional groups exposed to the solvent, as well as to the structural, dynamics and conformational properties. Amlodipine besilate, nimodipine, nitrendipine and nifedipine were applied in this investigation. All derivates are only sparingly soluble in water and therefore acetone, ethanol, dimethyl sulfoxide, and methylene chloride as solvents were chosen. The densities of solutions were measured at 25°C in the concentration range between 0.005 in 0.1 mol dm^{-3} and apparent molar volumes were calculated. Apparent molal volumes at infinity concentration, where only solute-solvent interactions could be assumed, were estimated. Obtained values are compared with those obtained by ACD/ChemSketch Program and Traube's additivity concept. Solvent properties were ascribed by three-component Hansen parameters and the dependence of volume properties on the hydrogen bonding and polar parameter was found.

Vpliv velikosti in topnosti molekul zdravilnih učinkovin na mehanizem sproščanja iz hidrofilnih ogrodnih tablet z različno velikostjo mrežnih por

V. Slameršek
Fakulteta za farmacijo, Univerza v Ljubljani, Slovenija
Mentorica: S. Baumgartner
Fakulteta za farmacijo, Univerza v Ljubljani, Slovenija

Hidrofilne ogrodne tablete predstavljajo sodobne farmacevtske oblike s prirejenim sproščanjem. Namen naše naloge je bil ugotoviti vpliv velikosti in topnosti molekul zdravilne učinkovine ter vpliv velikosti mrežnih por nabreklih polimerov na mehanizem sproščanja iz tovrstnih ogrodnih tablet. Kot modelne učinkovine smo uporabili diflunisal, pentoksifilin in vankomicinijev klorid. Osnovo ogrodnih tablet so tvorili visokomolekularni celulozni etri: hidroksietilceluloza (HEC), hidroksipropilceluloza (HPC) in hidroksipropilmetilcelulozi K4M in K100M (HPMC K4M, HPMC K100M). Tablete smo direktno stisnili, jim določili farmacevtsko tehnološke parametre, ter spremljali sproščanje učinkovin. Kinetiko in mehanizem sproščanja smo vrednotili po različnih teoretičnih modelih (Korsmeyer-Peppasov, Peppas-Sahlinov, Higuchijev in Hixon-Crowellov model). Ugotovili smo, da se dobro vodotopne učinkovine sproščajo z difuzijo, slabo vodotopne pa, če so majhne, s kombinacijo difuzije in erozije, nikakor pa ne le z erozijo. Potrdili smo, da manjše molekule oddifundirajo skozi pore nabreklega polimernega ogrodja hitreje in lažje kot večje molekule. Glede na velikost mrežnih por smo predvideli najhitrejše sproščanje učinkovin iz HEC tablet, sledilo naj bi sproščanje iz HPMC K4M, HPMC K100M in HPC tablet. Rezultati so potrdili naše napovedi za diflunisal in pentoksifilin. V primeru vankomicinijevega klorida pa je hitrost sproščanja nekoliko drugačna od napovedane, najverjetneje zaradi sočasnega vpliva različnih mehanizmov.

The Influence of the Drug Molecular Size and its Water Solubility on the Release Mechanism from Hydrophilic Matrix Tablets with Different Mesh Sizes

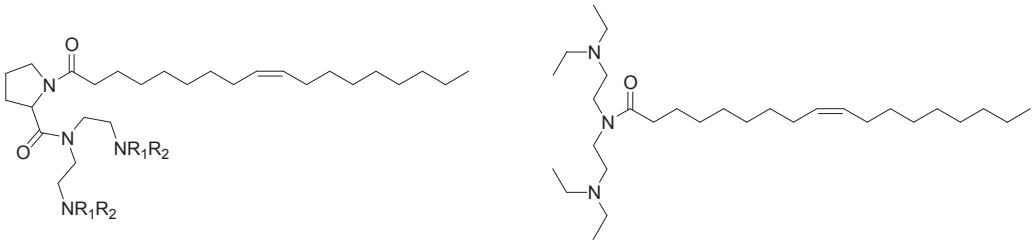
V. Slameršek
Faculty of Pharmacy, University of Ljubljana, Slovenia
Supervisor: S. Baumgartner
Faculty of Pharmacy, University of Ljubljana, Slovenia

Hydrophilic matrix tablets are modern modified release dosage forms. The purpose of this work was to investigate the influence of the drug molecular size and its solubility on the release mechanism from matrix tablets and the influence of different mesh sizes of swollen polymers on it. We used diflunisal, pentoxifylline and vancomycin hydrochloride as model drugs. High-molecular cellulose ethers: hydroxyethyl cellulose (HEC), hydroxypropyl cellulose (HPC) and hydroxypropyl methylcellulose K4M and K100M (HPMC K4M, HPMC K100M) were used as a hydrophilic polymers in formulation of matrix tablets. Matrix tablets were directly compressed, their physical and technological parameters were characterized and dissolution tests were carried out. Different dissolution models (Korsmeyer-Peppas, Peppas-Sahlin, Higuchi and Hixon-Crowell models) were applied to drug release data in order to evaluate the release mechanisms and kinetics. We found out that the release of freely water-soluble drugs was controlled by the diffusion, whereas a small water-insoluble drug was released by the combination of the diffusion and erosion, but never only by the erosion. It was proven that the diffusion of smaller molecules through the pores of swollen polymer matrix is faster and easier than the diffusion of bigger molecules. Regarding the mesh sizes the fastest drug release was predicted from HEC tablets followed by the release from HPMC K4M, HPMC K100M and HPC tablets. The results proved our predictions for diflunisal and pentoxifylline, whereas the release rate of vancomycin hydrochloride deviates from the expected ones, probably because of the simultaneous influences of different mechanisms.

Oleoil- in oleilamini kot potencialni anti-endotoksini

D. Šarlah
Kemijski inštitut, Ljubljana, Slovenija
Mentorica: A. Majerle
Somentorica: B. Mohar
Kemijski inštitut, Ljubljana, Slovenija

Sepsa je klinični sindrom, ki je posledica infekcije s patogenimi bakterijami in posledičnega telesnega odziva nanjo. Sprožijo jo komponente bakterijskih celičnih sten, predvsem endotoksini iz Gram-negativnih bakterij. Za zdravljenje sepse uporabljamo antibiotike, saj zaenkrat še ne poznamo uspešnejših zdravil. Zaradi vse večje odpornosti mikroorganizmov na antibiotike potekajo raziskave v smeri sinteze novih učinkovin. Mednje spadajo lipopoliamini, katerih določeni predstavniki učinkovito nevtralizirajo endotoksine.
V raziskovalnem delu je predstavljena sinteza nove skupine lipopoliaminov in testiranje njihovega antibakterijskega delovanja. Vse sintetizirane spojine vsebujejo polarno dietilentriaminsko skupino in hidrofoben oleoilni del. Pripravili smo tudi lipopoliamine, ki vsebujejo aminokislini L-prolin in L-lizin. Spojine kažejo podobno antibakterijsko delovanje kot peptidni antibiotik polimiksin B, ki je učinkovit anti-endotoksin. Dobljeni rezultati kažejo, da pripravljeni lipopoliamini predstavljajo dobro izhodišče za nadaljnje raziskave biološko aktivnih lipopoliaminov.



Oleoyl- and Oleylamines as Potential Anti-endotoxin Agents

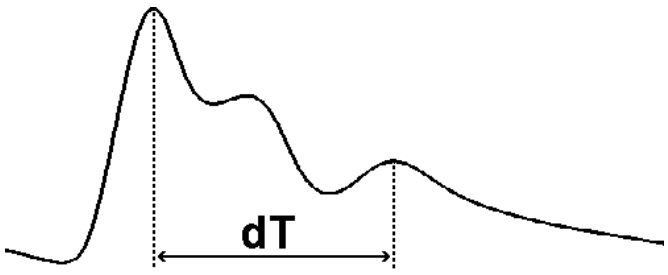
D. Šarlah
National Institute of Chemistry, Ljubljana, Slovenia
Supervisor: A. Majerle
Co-supervisor: B. Mohar
National Institute of Chemistry, Ljubljana, Slovenia

Sepsis is a clinical syndrome caused by infection with pathogenic bacteria and consequential response of immune system. It is triggered by components of bacterial cell wall, first of all endotoxins from Gram-negative bacteria. Antibiotics are used for treating sepsis in spite of their limited efficiency, and research for better alternatives is continuing due to increased bacterial resistance. Interestingly, it was found that some lipopolyamines neutralize endotoxin agents.
In this work, various new lipopolyamines were synthesized and their antibacterial activity has been tested. The prepared compounds contain a polar diethylentriamino residue and a hydrophobic oleoyl moiety. New lipopolyamines were prepared containing an L-proline or an L-lysine residue. All tested compounds have shown similar antibacterial activity compared to polymyxin B, a peptide antibiotic, known to be an efficient anti-endotoxin agent. These results point out that such modified lipopolyamines constitute a good research base in the quest for biologically active lipopolyamines.

Increased Large Artery Stiffness may Predispose to Vasovagal Syncope

J. Šimek
General University Hospital, Prague, Czech Republic
Supervisor: J. Hradec
Charles University, Prague, Czech Republic

Purpose: The mechanisms leading to vasovagal syncope (VVS) are still poorly understood. Recent study demonstrated that increased arterial wave reflection was associated with the occurrence of syncope. This observation raises a question whether VVS is associated with increased large artery stiffness.
Methods: Finger arterial pressure (FAP) waveforms (Finapres) were obtained in 20 head-up tilt test positive subjects with recurrent VVS (13 women, aged 39.5 ± 13.7 years) and in 20 sex and age-matched healthy controls. Subjects with clinical evidence of cardiovascular disease other than VVS were not included into the study. Recordings were performed in the supine position after 15-min rest. Recently introduced index of large artery stiffness (SI) was calculated as a ratio of subject height and time delay (dT) between the systolic and diastolic peaks of the FAP waveform (see the figure). This index was shown to be closely correlated with carotid-to-femoral pulse wave velocity. Both groups were compared by unpaired t-test.
Results: Compared to the control group, patients with VVS had significantly higher SI (5.95 ± 0.76 m/s vs. 5.19 ± 0.4 m/s, p = 0.00033). This difference remained significant (p = 0.006) after controlling for body mass index, mean heart rate, and mean arterial blood pressure (ANCOVA).
Conclusions: Our results support the hypothesis that increased large artery stiffness is a powerful predisposing factor for vasovagal syncope. This observation might have wider clinical implications, because the time delay between the systolic and diastolic peaks can be measured simply and rapidly by inexpensive photoplethysmograph devices.



Oblikovanje programa zvestobe na primeru podjetja Krka – Program kozmetika

Ž. Šošter
Ekonomsko-poslovna fakulteta, Univerza v Mariboru, Slovenija
Mentor: B. Završnik
Ekonomsko-poslovna fakulteta, Univerza v Mariboru, Slovenija
Somentorica: S. Verstovšek
Krka, d. d., Novo mesto, Slovenija

Na razvitih trgih ni dovolj le pridobivati nove kupce, temveč jih je potrebno tudi ohranjati. Da so stalni kupci za podjetje neprecenljiva vrednost in da jih mora podjetje čim bolje spoznati, se zaveda tudi Krka, d. d. Prav zaradi tega je namen naloge predstaviti način, s katerim bodo v podjetju uresničili svoje zastavljene cilje.
V nalogi sem skušala čim bolj raziskati in s praktičnimi primeri utrditi ustreznost oblikovanja programa zvestobe za kupce, ki je odličen način pridobivanja in ohranjanja zvestih kupcev. Prvi del vsebuje opise tržnega komuniciranja različnih avtorjev ter oblikovanje programov zvestobe in klubov. Začetek drugega dela opisuje dejavnosti podjetja Krka, d. d. in njegovega Programa Kozmetike. Najzanimivejši in najpomembnejši del naloge pa vsebuje opis načrtovanja programa zvestobe kupcev za Program Kozmetika, poimenovanega Klub Krka Kozmetika. Svojo zamisel o klubu sem izoblikovala tako, da sem najprej določila cilje, ciljno skupino, obliko kluba ter nato še klubske ugodnosti, finančni načrt, komunikacijsko strategijo kluba in temelje za oblikovanje podatkovne baze o članih kluba. Za večjo razpoznavnost kluba, sem nekaj besed namenila tudi oblikovni podobi kluba ter oblikovala obrazec za včlanitev v Klub Krka Kozmetika.
Oblikovanje programa zvestobe ni enostavno, vendar s pravim pristopim si je prav s tem marketinškim orodjem mogoče priboriti prednost pred ostalimi. Prav gotovo namreč velja, da je vsak zvesti kupec najboljše varovalo pred konkurenco.

Forming a Customer Loyalty Programme on the Case of the Krka Company – The Programme Cosmetics

Ž. Šošter
Faculty of Economics and Business, University of Maribor, Slovenia
Supervisor: B. Završnik
Faculty of Economics and Business, University of Maribor, Slovenia
Co-supervisor: S. Verstovšek
Krka, d. d., Novo mesto, Slovenia

On developed markets it is not enough just to gain new customers, but it is also necessary to keep them. The Krka. Company is also aware of the fact that the permanent customers are invaluable for the company and that the company has to know them as much as possible. For that reason the purpose of this project is to represent the way which will help the company to realize the established aims.
In the project I have tried to research and with the help of case studies to fortify the suitability of forming the Customer Loyalty Programme, which is an excellent way to gain and keep loyal customers. The first part contains the description of marketing communication by different authors and the forming of Customer Loyalty Programmes and Clubs. The beginning of the second part describes activities of the Krka Company and its Cosmetics Programme. Most interesting and most important part of the project contains description of setting up a Customer Loyalty Programme of the Cosmetics Programme, called Krka Cosmetics Club. I have formed own idea of the club so that I have first established goals, target group, the type of Loyalty programme and then the Loyalty programme benefits, the financial concept, strategy of communication of the loyalty programme and the foundations to form the Loyalty programme database. For bigger recognition of the programme, I have also destined a few words to the loyalty programme's formative image, and I have formed a blank form for affiliation to Krka Cosmetics Club.
Forming of Customer Loyalty Programme is not an easy task, but with the right accession it is possible to get the advantage of the others with this market tool. For it surely holds true, that loyal customer is the best factor of safety against the competition.

Patentno-pravno varstvo in njegov pomen za farmacevtsko industrijo

J. Špiler
Ekonomska fakulteta, Univerza v Ljubljani, Slovenija
Mentor: K. Puharič
Ekonomska fakulteta, Univerza v Ljubljani, Slovenija

Patent je za farmacevtsko industrijo najpomembnejši predmet pravnega varstva intelektualne lastnine, saj priznava originalnemu zdravilu monopol, ki ob pravilni poslovni strategiji omogoča imetnikom patenta povrnitev stroškov raziskav in razvoja ter ustvarjanje dobičkov. To vzpodbuja farmacevtske proizvajalce v raziskave in razvoj vedno novih, učinkovitejših zdravil, od česar imajo koristi farmacevtski proizvajalci (ustvarjanje novih dobičkov), država (hitrejši gospodarski razvoj) in bolniki.
Sporazum o trgovinskih vidikih pravic intelektualne lastnine (TRIPS) naj bi pripomogel k zmanjšanju ovir v mednarodni trgovini, upošteval potrebo po spodbujanju učinkovitega in ustreznega varstva pravic intelektualne lastnine ter istočasno poizkušal zagotoviti, da spoštovanje pravnega varstva intelektualne lastnine ne bi predstavljalo dodatne ovire v mednarodni trgovini.
Zaradi podaljševanja časa od odkritja oziroma iznajdbe nove aktivne učinkovine do pridobitve dovoljenja za prodajo in trženje novega zdravila je večina razvitih držav uvedla dopolnilne zakone oziroma predpise, ki omogočajo podaljšanje pravic iz osnovnega patenta zdravil.
Vzporedno s podaljševanjem pravic iz osnovnega patenta pa želijo države članice WTO-ja omogočiti dostop novih in učinkovitejših zdravil širšemu krogu bolnikom (velika večina Afričanov si ne more privoščiti dragih originalnih zdravil). Tako TRIPS dovoljuje omejene izjeme od pravic iz patenta in omogoča državam pod določenimi pogoji (na primer: epidemija AIDS-a v Afriki) podelitev prisilne licence. Država z njo uradno dovoli prodajo cenejšega generičnega zdravila, kljub veljavnosti patentno-pravnega varstva originalnega zdravila.

Patent Protection and its Significance for Pharmaceutical Industry

J. Špiler
Faculty of Economics, University of Ljubljana, Slovenia
Supervisor: K. Puharič
Faculty of Economics, University of Ljubljana, Slovenia

A patent is the most important item of intellectual property protection in pharmaceutical industry since it respects the monopoly of the original drug which, with a right strategy, enables patent holders the reimbursement of their costs of research and development and creates profit. This incites pharmaceutical producers to develop new, more effective drugs giving benefit to the producers (creating new profits), the state (rapid economical development) and to the patients.
Trade Related Aspects of Intellectual Property Rights (TRIPS) should remove the obstacles in international trade, consider the need to encourage an effective and appropriate intellectual property protection and at the same try to ensure respect of legal protection of the intellectual property without negative effect on the international trade.
The majority of developed countries introduced bylaws or rules in order to prolong the period from the invention of a new active substance till the acquisition of marketing authorization for a new drug. These bylaws enable prolongation of rights arising from the basic drug patent.
Concomitantly with prolongation of the rights from the basic drug patent, the WTO members wish to give a wider population of patients the access to newer and more effective drugs (the majority of African people cannot afford expensive original drugs). Thus TRIPS allows the limited exceptions to the patent rights and under certain conditions (eg epidemics of AIDS in Africa) enables some countries to grant a compulsory licence for a generic drug. Thus the country officially allows the sales of a less expensive generic drug during the validity of patent protection of the original drug.

Novi katalizatorji za pripravo kiralnih farmacevtskih intermediatov

D. Šterk
Kemijski inštitut, Ljubljana, Slovenija
Mentorica: B. Mohar
Kemijski inštitut, Ljubljana, Slovenija

Asimetrično transfer hidrogeniranje je pomembna metoda za pripravo kiralnih sekundarnih alkoholov. Do sedaj so bili doseženi najboljši rezultati z Noyorijevim kiralnim Ru(II)-*N*-(tozil)difeniletilendiamin kompleksom.
Pripravili smo nove *N*-arilsulfonil-1,2-diaminske ligande izhajajoč iz (1*S*,2*S*)-difeniletilendiamina. Lastnosti ligandov smo testirali na Ru(II) kataliziranem asimetričnem transfer hidrogeniranju na različnih vrstah ketonov.
Novi Ru(II)-(1*S*,2*S*)-*N*-(arilsulfonil)difeniletilendiamin kompleksi so pokazali enako ali boljšo stereoselektivnost kot Noyorijev katalizator. Posebno sta izstopala kompleksa z (1*S*,2*S*)-*N*-(2,4,6-triizopropilbenzensulfonil) -1,2-difeniletilendiaminom in *N*,*N'*-bis[(1*S*,2*S*)-2-amino-1,2-difeniletil)]benzen-1,2-disulfonamidom, ki sta dala najvišje enantioselektivnosti pri redukciji metil benzoilformata, etil benzoilformata, 1,1,1-trifluoro-3-fenilpropan-2-ona in 2-(trifluorometil)acetofenona z uporabo HCO_2H-Et_3N azeotropa.

Novel Catalysts for the Preparation of Pharmaceutical Intermediates

D. Šterk
National Institute of Chemistry, Ljubljana, Slovenia
Supervisor: B. Mohar
National Institute of Chemistry, Ljubljana, Slovenia

Asymmetric transfer hydrogenation is an attractive method for preparing chiral secondary alcohols. Up to date the best results were obtained using Noyori's chiral Ru(II)-*N*-(tosyl)diphenylethylenediamine complex.
We prepared novel *N*-arylsulfonyl-1,2-diamine ligands derived from (1*S*,2*S*)-diphenylethylenediamine. The properties of these ligands were explored in Ru(II) catalysed transfer hydrogenation of various classes of ketones.
The new Ru(II)-(1*S*,2*S*)-*N*-(arylsulfonyl)diphenylethylenediamine complexes showed equal or even better stereoselectivity than Noyori's catalyst. In particular, the complexes with (1*S*,2*S*)-*N*-(2,4,6-triisopropyl-benzenesulfonyl)-1,2-diphenylethylenediamine or *N*,*N'*-bis[(1*S*,2*S*)-2-amino-1,2-diphenylethyl)]benzene-1,2-disulfonamide proved to give the highest enantioselectivities in the transfer hydrogenation of methyl benzoylformate, ethyl benzoylformate, 1,1,1-trifluoro-3-phenylpropan-2-one and 2-(trifluoromethyl) acetophenone using HCO_2H-Et_3N azeotrope.

Uporaba IR spektroskopije pri spremljanju kristalizacijskega procesa felodipina in nifedipina

A. Šuštarič
Fakulteta za farmacijo, Ljubljana, Slovenija
Mentor: S. Srčič
Somentorica: A. Zega
Fakulteta za farmacijo, Ljubljana, Slovenija

Infrardeča spektroskopija je ena izmed metod, s katero lahko opazujemo razlike med kristalno in amorfno obliko ter prehod ene oblike v drugo tako na kvalitativni kot tudi na kvantitativni ravni.
Preiskovali smo felodipin in nifedipin, ki sta kalcijeva antagonista. Na začetku dela smo z analizo zmesi, sestavljenih s felodipinom oziroma nifedipinom in mikrokristalno celulozo, poskušali ugotoviti, katera infrardeča tehnika je najbolj primerna za analizo vzorcev v obliki praškov. S statistično metodo regresije večdimenzionalnih podatkov na osnovi vsote najmanjših kvadratov, ki je primerna za obdelavo kompleksnih IR spektrov, smo izračunali koeficient korelacije in standardno deviacijo kalibracijske metode. Na osnovi tega smo se odločili, da je najprimernejša IR tehnika razpršena odbojnost (DR) v bližnjem infrardečem območju.
S tehniko razpršene odbojnosti v bližnjem infrardečem območju smo analizirali tudi tablete, ki so vsebovale obe obliki učinkovine. Potrdili smo, da je to edina metoda, pri kateri ni potrebna nikakršna predpriprava tablet za analizo, saj je tehnika neinvazivna in nedestruktivna. Kakršnakoli predpriprava tablet pred analizo je namreč povezana s spremembami v fizikalnem stanju preiskovanih snovi.

Use of IR Spectroscopy for Monitoring Crystallization Process of Felodipine and Nifedipine

A. Šuštarič
Faculty of Pharmacy, Ljubljana, Slovenia
Supervisor: S. Srčič
Co-supervisor: A. Zega
Faculty of Pharmacy, Ljubljana, Slovenia

Infrared spectroscopy is one of the methods used for monitoring the differences between crystalline and amorphous forms and the transition of one form into another on a qualitative as well as quantitative level.
In our research work we investigated felodipine and nifedipine, which are calcium antagonists. At the beginning we tried to find, by analyzing the compounds composed of felodipine or nifedipine and microcrystalline cellulose, the most convenient infrared technique for analyzing samples in the powder state. By using the statistical method of partial least squares algorithm, which is convenient for complex IR spectra processing, the correlation coefficient and the standard deviation of calibration method were calculated. On that basis we found the IR technique of diffuse reflectance (DR) in near infrared range the most convenient method.
By using the technique of diffuse reflectance in near infrared range we analyzed also tablets containing ingredients in both states. We confirmed that this is the only method, which does not require any pre-preparation of tablets for analysis because it is completely noninvasive and nondestructive technique. Namely, any preparation of tablets prior to analysis is always connected with certain changes in physical state of investigated substances.

Bioadhezija mikrosfer iz zmesi polikarbofila in hitosana na sluznico sečnega mehurja prašiča

J. Trontelj, P. Veržun
Fakulteta za farmacijo, Univerza v Ljubljani, Slovenija
Mentorica: M. Bogataj
Fakulteta za farmacijo, Univerza v Ljubljani, Slovenija

UVOD: Bioadhezija je dogajanje, med katerim se naravni ali sintetični polimer prilepi (adherira) na biološko komponento, običajno na sluznico v telesu. V tej nalogi smo preučevali vpliv razmerja dveh bioadhezivnih polimerov, hitosanijevega klorida (CH) in polikarbofila (PC) na adhezijo mikrosfer in polimernih filmov iz teh snovi na sluznico izoliranega sečnega mehurja prašiča. CH je polimer, ki nosi pozitivni, PC pa negativni naboj. Če nastopata oba polimera v zmesi, lahko pride med njima do interakcij, kar vpliva na njune lastnosti, pomembne za bioadhezijo.
METODE: Za ugotavljanje deleža adhezije mikrosfer smo uporabili model celega izoliranega sečnega mehurja. Za določitev jakosti bioadhezije pa smo uporabili tenziometrično metodo, s katero smo merili silo ločevanja polimernega filma od koščka sluznice sečnega mehurja. Hitrost nabrekanja mikrosfer smo določali z merjenjem volumna tekočine, ki jo absorbirajo mikrosfere, stisnjene v tableto.
REZULTATI: Polimernim filmom z utežnim razmerjem CH : PC = 50 : 50 smo izmerili najmanjšo silo ločevanja. Nekoliko nepričakovano smo pri istem utežnem razmerju ugotovili največji delež adheriranih mikrosfer. Mikrosfere s tem razmerjem obeh polimerov so nabrekale le z zmerno hitrostjo.
SKLEP: Na proces adheriranja mikrosfer vpliva več faktorjev. Kinetika nabrekanja polimerov je v opazovanem primeru močneje vplivala na obseg adhezije mikrosfer, kot pa sama jakost bioadhezivne vezi, ki se vzpostavi v naslednji fazi.

Bioadhesion of Polycarbophil/Chitosan Microspheres to the Porcine Urinary Bladder Mucosa

J. Trontelj, P. Veržun
Faculty of Pharmacy, University of Ljubljana, Slovenija
Supervisor: M. Bogataj
Faculty of Pharmacy, University of Ljubljana, Slovenija

INTRODUCTION: Bioadhesion is a process in which a synthetic or natural polymer adheres to a biological component such as mucosa. In this study we examined bioadhesion of microspheres and polymeric films prepared from two bioadhesive polymers, chitosan hydrochloride (CH) and polycarbophil (PC), to the mucosa of the porcine urinary bladder as a function of ratio between the two polymers. CH is a cationic, whereas PC is an anionic polymer. If both polymers are used in a mixture interactions between them will occur. These interactions may affect polymers' properties important for bioadhesion.
METHODS: Bioadhesion of microspheres was evaluated on isolated pig's urinary bladder. Bioadhesion strength of polymeric films was assessed by measurements of the detachment force required to separate the polymeric films from bladder mucosa. The kinetics of microsphere swelling was determined by measuring the volume of liquid absorbed by the tablets formed from compressed microspheres.
RESULTS: Polymeric films with PC : CH ratio of 50 : 50 showed the weakest detachment force. Somewhat unexpectedly, at the same ratio, the largest fraction of adhered microspheres was found. The mixture with this ratio displayed only a moderate swelling rate.
CONCLUSION: Bioadhesion of microspheres is influenced by several factors. In the studied situation, the extent of microsphere adhesion was influenced more strongly by kinetics of swelling than by the strength of bioadhesion bond which is subsequently formed.

Aktivna sekrecija transportnega markerja fluoresceina skozi steno tankega črevesa podgane

D. Uršič
Fakulteta za farmacijo, Univerza v Ljubljani, Slovenija
Mentor: A. Kristl
Fakulteta za farmacijo, Univerza v Ljubljani, Slovenija

Fluorescein se uporablja kot marker za pasivni paracelularni transport, s pomočjo katerega pri poskusih določanja permeabilnosti skozi izoliran segment intestinalnega tkiva poskusnih živali in vitro spremljamo integriteto tkiva. V predhodnih raziskavah se je izkazalo, da fluorescein ob prisotnosti glukoze na mukozni strani prehaja s serozne na mukozno stran tudi z aktivnim transportom. To aktivno sekrecijo pripisujemo t.i. MRP(multidrug resistance associated protein) transporterju. Potrdili smo, da glukoza poveča sekrecijo fluoresceina s serozne na mukozno stran in dokazali, da fruktoza, galaktoza in α-metil-D-glukopiranozid nimajo enakega vpliva na transport fluoresceina kot glukoza. Pokazalo se je tudi, da ibuprofen, znana NSAID učinkovina, dodatno pospeši transport fluoresceina s serozne na mukozno stran, ko je glukoza prisotna na mukozni strani. Izvedli smo dva poskusa z aciklovirjem in ugotovili, da se tudi transport aciklovirja poveča ob dodatku glukoze na mukozno stran in da je transport najverjetneje povezan s transportom fluoresceina.

Active Secretion of Transport Marker Fluorescein across Rat Small Intestine

D. Uršič
Faculty of Pharmacy, University of Ljubljana, Slovenia
Supervisor: A. Kristl
Faculty of Pharmacy, University of Ljubljana, Slovenia

Fluorescein is used as a marker for passive paracellular transport. With fluorescein tissue integrity is followed in in vitro experiments for determination of permeability across isolated segment of guinea-pig intestinal tissue. In preliminary studies it was shown that when glucose is present on mucosal side of the tissue, fluorescein passes from serosal to mucosal side with active transport, too. This active secretion is attributed to MRP (multidrug resistence associated protein) transporter. We have confirmed that glucose inceases secretion fluorescein from serosal to mucosal side and proved that fructose, galactose and α-methyl-D-glucopyranoside do not have the same influence on transport of fluorescein as glucose does. It was also shown that ibuprofen, known NSAID drug, additionally increases transport of fluorescein from serosal to mucosal side when glucose is present on mucosal side. We have also performed two experiments with acyclovir and have established that transport of acyclovir is also increased when glucose is added to mucosal side and that transport of acyclovir in most probably linked to fluorescein transport.

Detekcija in vezava endotoksina na proteine

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

Endotoksin ima pomembno vlogo v patofiziologiji Gram-negativne sepse in lahko vodi v septični šok. Za pravočasno zdravljenje je pomembna hitra detekcija endotoksina v krvi. Njegovo prisotnost v krvi z običajnimi metodami, kot je amebocitni lizat iz *limulusa* (LAL-test), težko zaznamo, ker se lahko veže na številne plazemske in celične proteine, ki ga zamaskirajo.
Odkrili smo, da protein MRP, ki se nahaja v citosolu, močno veže endotoksin. Rekombinanten MRP smo pridobili v *E. coli* ter ga izolirali. Pred izvedbo poskusov, s katerimi smo analizirali vezavo endotoksina na MRP, je bilo potrebno iz proteinskih vzorcev odstraniti endotoksin. Za odstranjevanje endotoksina od rekombinantnega proteina sta se kot najboljši metodi izkazali afinitetna kromatografija oziroma ekstrakcija s sledečo RP-HPLC. Vezavo endotoksina na MRP smo pokazali s fluorescenco in ELISA testom.
Dejansko koncentracijo endotoksina smo v prisotnosti proteinov, ki ga vežejo, določili z encimsko razgradnjo kompleksa protein-endotoksin. Encimsko razgradnjo rekombinantnega MRP, lizocima in serumskih proteinov smo izvedli s proteinazo K in z LAL-testom določili absolutno količino endotoksina. Tako modificiran LAL-test obeta uspešnejšo detekcijo endotoksina v krvi pacientov s sepso.
Ugotovili smo tudi, da lahko termična denaturacija proteina endotoksin dodatno zamaskira, saj se ob denaturaciji izpostavijo hidrofobni deli iz sredice proteina, ki vežejo endotoksin. Na osnovi tega lahko sklepamo, da so številne raziskave, v katerih so poročali, da proteini sprožijo enako signalno pot kot endotoksin, napačne. MRP za razliko od drugih proteinov nima hidrofobne sredice, zato pri njem ob termični denaturaciji ne pride do dodatnega zamaskiranja endotoksina.

Detection and Binding of Endotoxin to Proteins

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

Endotoxin has an important role in the pathophysiology of Gram-negative sepsis and can lead to septic shock. Early detection of the endotoxin in the blood is imperative for successful treatment. Presence of endotoxin in the blood with standard methods like LAL-assay is difficult to detect as it may bind to numerous plasmatic and cell proteins that mask it.
It was detected that endotoxin strongly binds to the protein MRP, which is found in cytosol. Recombinant MRP was expressed in *E. coli* and isolated. Before conducting the experiments which would show binding of endotoxin to the MRP, it was necessary to remove the endotoxin from the protein samples. Removal of endotoxin from these samples was best achieved by either affinity chromatography or extraction followed by RP-HPLC. The binding of the endotoxin to the protein MRP was shown with the fluorescence spectroscopy and ELISA.
The detection of endotoxin was improved with enzyme degradation of the protein-endotoxin complex even with the binding proteins present. Enzyme degradation of recombinant MRP, lysozyme and serum proteins was achieved with proteinase K and the absolute quantity of endotoxin was measured with the LAL-test. This modified LAL-test enables more successful detection of endotoxin in blood of patients with the sepsis.
It was found that thermic denaturation could further mask the endotoxin as hydrophobic regions of protein that bind endotoxin are exposed from the core of the protein during denaturation. From this it can be concluded that research previously conducted, where it was suggested that proteins trigger same signalling pathway like endotoxin were incorrect. MRP is different from the other proteins as it does not have hydrophobic core therefore it does not thermally mask the endotoxin.

Razvoj presejalnega testa za določanje mutacij v genu *HFE* s tehnologijo TaqMan®

T. Vaupotič
Zavod RS za transfuzijsko medicino, Ljubljana, Slovenija
Mentorica: V. Čurin Šerbec
Somentorica: R. R. Rupreht
Zavod RS za transfuzijsko medicino, Ljubljana, Slovenija

Dedna hemokromatoza (DH) je pogosta genetska bolezen metabolizma železa, za katero je značilno prekomerno kopičenje železa v jetrih, pankreasu in srčni mišici. Neodkrita DH povzroči cirozo jeter, hepatocelularni karcinom in diabetes. Med pripadniki bele rase je frekvenca DH med 1:200 in 1:400. Povzročajo jo predvsem tri mutacije v eksonih gena *HFE*: 187C→G (H63D), 193A→T (S65C) in 845G→A (C282Y). V okviru raziskovalne naloge smo razvili zanesljiv test za določanje mutacij 187C→G, 193A→T in 845G→A, ki temelji na PCR v realnem času z uporabo tehnologije TaqMan[å]. Za učinkovito alelno diskriminacijo vseh treh polimorfizmov sta potrebna samo dva para začetnih oligonukleotidov in trije pari fluorescenčnih MGB sond. Rezultati genotipizacije s tehnologijo TaqMan[å] so se popolnoma ujemali z rezultati določanja zaporedja nukleotidov DNA in analizo RFLP. Metoda je primerna tako za presejalni test kot za rutinsko molekularno diagnostiko, saj ne zahteva nadaljnjih post-PCR postopkov. Uporabili jo bomo za določitev frekvenc omenjenih mutacij v Sloveniji.

Development of TaqMan® Assay for *HFE* Gene Mutations Screening

T. Vaupotič
Blood Transfusion Centre of Slovenia, Ljubljana, Slovenia
Supervisor: V. Čurin Šerbec
Co-supervisor: R. R. Rupreht
Blood Transfusion Centre of Slovenia, Ljubljana, Slovenia

Hereditary hemochromatosis (HH) is an autosomal recessive disorder of the iron metabolism. Affected individuals chronically absorb elevated amounts of iron from the diet, resulting in accumulation of excess iron mainly in the liver, pancreas and heart. If untreated, HH leads to cirrhosis, hepatocellular carcinoma and diabetes. Estimated HH frequency in the Caucasian population is between 1:200 and 1:400. The most prevalent mutations in HH are 187C→G (H63D), 193A→T (S65C) and 845G→A (C282Y) in the *HFE* gene. We developed an accurate real-time PCR assay for genotyping the 187C→G, 193A→T and 845G→A mutations based on TaqMan[å] technology. Only two primer pairs and three pairs of sequence specific fluorescent MGB probes are required for efficient allele discrimination of all three SNP's. We compared TaqMan[å] genotyping results with the RFLP method and DNA sequencing and there were no discrepancies. This method is not only suitable for large-scale genetic screening but also for routine molecular diagnostic as it requires no post-PCR handling. It will be used to study the frequency of the three main mutations in the Slovene population.

Izolacija glavnih komponent bacitracina s preparativno tekočinsko kromatografijo visoke ločljivosti (HPLC) in ekstrakcijo v trdni fazi (SPE)

T. Vehovec
Krka, d. d., Novo mesto, Slovenija
Mentor: V. Kmetec
Fakulteta za farmacijo, Univerza v Ljubljani, Slovenija
Somentor: V. Pavli
Krka, d. d., Novo mesto, Slovenija

Peptidi in proteini so vedno bolj pogosti biofarmacevtski produkti, katerih čistota je zelo pomembna za njihovo kakovost, učinkovitost in varnost. Trenutno je preparativna HPLC tehnika najbolj uporabna in vsestranska metoda za izolacijo čistih aktivnih substanc v farmacevtski industriji. Kot eno od modelnih molekul za peptidne substance lahko smatramo bacitracin (Bc) s peptidno strukturo in kompleksno sestavo. Opisali smo optimalen pristop pri razvoju zahtevne preparativne separacije Bc na koloni Kromasil C-8, 5µm (250 x 16mm i.d.). Po izolaciji na preparativni HPLC koloni smo izvedli odstranitev soli pufra, koncentriranje eluatov s posameznimi komponentami Bc s pomočjo ekstrakcije v trdni fazi (SPE) ter na koncu osušitev z liofilizacijo. Med posameznimi izolacijskimi koraki smo izvajali kontrolo učinkovitosti čiščenja eluatov. Sorodne substance Bc smo kontrolirali s hitro HPLC metodo na monolitni silikagelski Chromolith koloni in učinkovitost odstranjevanja ostankov soli fosfatnega pufra na SPE koloni z uporabo dokaznega reagenta za kalijeve ione (kalignost). Kvantitativen izkoristek izolacije posameznih komponent Bc je bil relativno nizek (med 45% in 83% za posamezne komponente), predvsem zaradi specifičnega pristopa pri zbiranju eluatov, da smo zagotovili visoko čistoto posameznih komponent. Čistoto posameznih izoliranih komponent Bc smo preverjali kromatografsko z »diode array« detekcijo in potrdili, da ni prihajalo do interferenc z njihovimi sorodnimi substancami.

Isolation of Main Bacitracin Components by High Performance Liquid Chromatography (HPLC) and Solid Phase Extraction (SPE)

T. Vehovec
Krka, d. d., Novo mesto, Slovenia
Supervisor: V. Kmetec
Faculty of Pharmacy, University of Ljubljana, Slovenia
Co-supervisor: V. Pavli
Krka, d. d., Novo mesto, Slovenia

Peptides and proteins are more and more frequent biopharmaceutical products, which purity is very important for their quality, efficiency and safety. Preparative HPLC is currently the most applicable and universal method for isolation of pure active substances in pharmaceutical industry. As one of the representative molecules for various protein drugs can be considered bacitracin (Bc) with peptide structure and complex composition. We described an optimal approach in the development of complex preparative separation of Bc using the column Kromasil C-8, 5 µm, (250 x 16mm i.d.). After the isolation on the preparative HPLC column we removed buffer from the eluates with individual components of Bc, concentrated them by solid-phase extraction (SPE) and dried them with lyophilisation. During isolation steps we controlled efficiency of eluate's purity. Related substances were controlled with the fast HPLC method on the monolith silicagel Chromolith column and residuals of the salt, which remained from phosphate buffer after SPE, with the reagent for potassium ions (calignost). The quantitative recovery of individual Bc components was relatively low (between 45 % and 83 % for individual component), mostly because of specific approach in collecting eluates, with which the high purity of active components was ensured. The purity of isolated components of Bc were verified chromatographically with diode array detection and confirmed the absence of interferences with their related substances.

Protokol endokrinoloških testiranj športnikov pri ugotovljenem povišanem razmerju testosterona proti epitestosteronu v urinu

K. Vernig
Fakulteta za farmacijo, Univerza v Ljubljani, Slovenija
Mentor: J. Osredkar
Fakulteta za farmacijo, Univerza v Ljubljani, Slovenija

Anabolični androgeni steroidi so sintetični derivati moškega spolnega hormona testosterona. Mednarodni olimpijski komite smatra uporabo androgenih anaboličnih steroidov kot doping. Za kontrolo zlorabe testosterona sta Donike in Zimmermann uvedla pojem razmerja testosteron/epitestosteron (T/E razmerje). Pokazalo se je, da se razmerje poveča po vnosu testosterona v telo. Če je razmerje T/E v urinu manjše od 6, je rezultat negativen. Kadar se vrednosti gibljejo med 6 in 10, lahko opravimo dodatna testiranja. Pozitivni rezultat je, če je razmerje večje kot 10.
Obravnavali smo primer športnika, pri katerem je bilo ugotovljeno povišano razmerje T/E in je bila vrednost količnika med 6 in 10. Opravili smo nadaljnje preiskave. Športniku smo v tridnevni preiskavi devetkrat vzeli kri in mu tekom treh dni zbirali urin. Drugi dan smo mu peroralno aplicirali ketokonazol, ki povzroči v telesu manjšo steroidogenezo na osnovi blokade citokrom P450 encimskega sistema. V kolikor razmerje T/E po testu s ketokonazolom ostane povišano in s tem tudi koncentracija testosterona v urinu oz. krvi, pomeni, da je bil testosteron v telo vnesen. V naši preiskavi smo naredili potrebne analize v urinu in krvi. Ugotovili smo, da se je drugi dan preiskave koncentracija testosterona tako v krvi kot tudi v urinu zmanjšala. S tem je sledil tudi padec razmerja T/E. Na ta način smo pokazali, da povišano razmerje ni posledica vnosa androgenih anaboličnih steroidov.

Endocrinological Testing of Athletes in the Case of Higher Testosterone Epitestosterone Ratio in Urine

K. Vernig
Faculty of Pharmacy, University of Ljubljana, Slovenia
Supervisor: J. Osredkar
Faculty of Pharmacy, University of Ljubljana, Slovenia

Androgenic anabolic steroids are in fact synthetic derivatives of the male sex hormone testosterone. The International Olympic Committee alike considers using anabolic steroids doping. In order to control the abuse of testosterone Donike and Zimmermann introduced a new term: a testosterone/epitestosterone ratio (T/E ratio). In human body this ratio is increased only after testosterone's application into the body. When the T/E ratio in urine is lower than 6, the result is considered negative. When the values vary between 6 and 10, further tests can be performed. When the ratio is higher than 10, the result is positive.
We considered the example of an athlete with an increased T/E ratio with a value between 6 and 10. Further tests were performed. During the three-day investigation his blood was taken nine times and urine was regularly collected. On the second day ketoconazole was perorally applied. Ketoconazole in human body causes a minor steroidogenesis based on a blockade of the cytochrom P450 enzyme system. In the case that T/E ratio and thus the concentration of testosterone in urine and blood remain higher, it is obvious that testosterone was introduced into the body. In the process of our investigation we performed the necessary analyses of urine and blood. We determined that on the second day of our investigation the concentration of testosterone in blood and urine was reduced. What followed was the reduction of the T/E ratio. Thus we proved that the increased ratio was not a consequence of the aplication of androgenic anabolic steroids.

Finančni in davčni učinki poslovanja in razvoja multinacionalnega podjetja v farmacevtski panogi

M. Vidmar Berus
Krka, d. d., Novo mesto, Slovenija
Mentor: L. Repovž
Somentor: M. Jurše
Ekonomsko-poslovna fakulteta, Univerza v Mariboru, Slovenija
Somentor: B. Kastelec
Krka, d. d., Novo mesto, Slovenija

Nosilci procesa internacionalizacije so multinacionalne družbe, ki utrjujejo svoj položaj na pravilno izbranih trgih z mednarodno razvejeno mrežo podružnic v tujini. Danes podjetje brez intenzivne internacionalizacije ne more (p)ostati konkurenčno, še zlasti pa to velja za družbe iz majhnih gospodarstev, med katere spada tudi Slovenija. Temeljni problem farmacevtske industrije danes je v naraščanju stroškov za raziskave, razvoj in trženje ob stalno rastočem tveganju razvojnega in tržnega neuspeha. Možnosti za razvoj novih zdravil se vse bolj zmanjšujejo zaradi skrajševanja zaščite intelektualne lastnine, ki omogoča doseganje dobičkov, potrebnih za povrnitev visokih razvojnih stroškov. S tem se pojavlja izrazita delitev farmacevtske industrije na razvojno aktivna in generična podjetja. V zadnjih letih se poleg razvojno aktivnih podjetij – originatorjev – vse pogosteje strateško povezujejo tudi generični proizvajalci, ker zahtevane rasti ne morejo dosegati le z organsko rastjo, zato prevzemi in združitve postajajo eden osnovnih načinov rasti tudi za generične družbe. Proučili smo razloge za razvoj multinacionalnega podjetja ter njihovo poslovanje s poudarkom na financiranju, investiranju in obdavčitvi mednarodnih družb in njihovih podružnic ter na osnovi analize farmacevtske panoge izdelali model finančnega upravljanja multinacionalnega podjetja v farmacevtski panogi in ta model uporabili na primeru Krke, d. d., Novo mesto.

Financial and Tax Effects on Operation and Development of Multinational Company in Pharmaceutical Branch

M. Vidmar Berus
Krka, d. d., Novo mesto, Slovenia
Supervisor: L. Repovž
Co-supervisor: M. Jurše
The School of Business and Economics, University of Maribor, Slovenia
Co-supervisor: B. Kastelec
Krka, d. d., Novo mesto, Slovenia

The internationalization process is run by multinational companies, reinforcing their position on correctly chosen markets with a widely spread international network of subsidiaries abroad. Today, a company not involved in intensive internationalization cannot become/stay competitive, which is particularly true for smaller-scale economies, including Slovenia. The basic problem of the pharmaceutical industry today lies in increased research, development and marketing costs, along with higher risks associated with the development or marketing failure. The chances for developing new drugs are becoming scarce because of the reduction of intellectual property protection, which enables the companies to achieve profits, required for settling high development costs. This brings about a sharp division in the pharmaceutical industry into originators and generics. Therefore beside the originators their generic competitors also use strategic alliances more frequently than they used to, since they cannot reach their goals through organic growth only. Consequently, mergers and acquisitions are also used as one of the basic methods for achieving the growth of generics. In our thesis we have studied the reasons for the development of a multinational company and its business operations and have underlined the financing, investments and taxation of international companies and their subsidiaries. Having analyzed the pharmaceutical branch, we have elaborated a financial management model in a multinational company within the pharmaceutical branch and have applied this model to show the example of Krka, d. d., Novo mesto.

Vpliv vrste Eudragita in topila na izdelavo mikrosfer z metodo odparevanja topila

I. Virant, S. Zavrl
Fakulteta za farmacijo, Univerza v Ljubljani, Slovenija
Mentorica: T. Mateović
Fakulteta za farmacijo, Univerza v Ljubljani, Slovenija

Metoda priprave mikrosfer z odparevanjem topila omogoča modifikacijo številnih parametrov in na ta način pripravo mikrosfer določenih lastnosti. V naši raziskavi smo poskušali ugotoviti, kako na nastanek mikrosfer vpliva vrsta uporabljenega topila notranje faze in vrsta Eudragita. V ta namen smo izdelovali mikrosfere z metodo odparevanja topila v štirih topilih notranje faze: acetonu, etanolu, metanolu in etil acetatu. V vsakem topilu smo poskušali izdelati mikrosfere Eudragitov E, RS, L, S in FS, ki so pogosto uporabljani polimeri pri izdelavi mikrosfer z metodo odparevanja topila. Kot zunanjo fazo smo uporabili tekoči parafin. V primeru nastanka mikrosfer smo jim določili povprečne velikosti in širino distribucije velikosti ter mikrosfere po velikostih frakcijah pregledali z optičnim mikroskopom.
Rezultati kažejo, da so za nastanek zelo pomembne lastnosti topila. Pokazali smo, da velikost nastalih mikrosfer Eudragita E narašča s padanjem dielektrične konstante topila notranje faze, ki verjetno vpliva tudi na mehanizem nastanka mikrosfer. V etil acetatu nam pri izbranih pogojih mikrosfer ni uspelo izdelati, najverjetneje zaradi zelo nizke dielektrične konstante. Ugotovili smo, da na nastanek mikrosfer vpliva tudi narava uporabljenega Eudragita in stabilizatorja. Pokazali smo, da magnezijev stearat veliko bolje stabilizira emulzijo ob uporabi Eudragitov s kationskimi skupinami (E, RS) kot pri Eudragitih z anionskimi skupinami.

The Influence of Solvent and Eudragit Type on Microspheres Prepared by Solvent Evaporation Method

I. Virant, S. Zavrl
Faculty of Pharmacy, University of Ljubljana, Slovenia
Supervisor: T. Mateović
Faculty of Pharmacy, University of Ljubljana, Slovenia

The solvent evaporation method is a flexible procedure, which enables preparation of microspheres with desired properties. In this work we have evaluated the type of Eudragit as matrix polymer, and the type of inner phase solvent on microsphere preparation. We have used four different inner phase solvents – acetone, methanol, ethanol, and ethyl acetate. With each solvent six different Eudragits, frequently utilised for microspheres preparation, were tested – Eudragit E, RS, S, L, L-55, and FS. Liquid paraffine was used as an outer emulsion phase. Successfully isolated microspheres were examined by microscope and their average particle size and particle size distribution were determined.
The results show that the inner phase solvent strongly influences microspheres formation. Average particle size of Eudragit E microspheres increases as the solvent dielectric constant decreases. Ethyl acetate was found to be inappropriate solvent for applied preparative conditions. Our results also show that magnesium stearate stabilises emulsion more efficiently in the case of Eudragits with cationic functional groups (E, RS) than in the case of Eudragits with anionic functional groups.

Priprava fuzijskih genov, ki kodirajo za piruvat karboksilazne izoforme in zeleni fluorescentni protein

S. Vozelj
Fakulteta za farmacijo, Granada, Španija
Mentor: A. Mlinarič
Fakulteta za farmacijo, Univerza v Ljubljani, Slovenija
Somentor: A. Morales Vargas
Fakulteta za farmacijo, Granada, Španija

Piruvat karboksilaza (PC) je metabolni encim, ki katalizira pretvorbo piruvata v oksalacetat. Razumevanje kontrole nadzora izražanja genov je ena izmed glavnih nalog moderne molekularne biologije
Promotorja Piruvatne karboksilaze P1 (proksimalni) in P2 (distalni) smo klonirali v pEGFP-N2 vektor.
Drugi del poskusa je zajemal transfekcijo evkarjontskih celic (NRK-normal rat kidney) z reagentom, ki deluje na osnovi liposomskega prenosa dednega materiala v celico. Uporabljali smo pEGFP-N_2 vektor, v katerega so bili predhodno vstavljeni različni konstrukti (opisani v tabeli št. 5.8) dednega materiala. Fluoriscirajoči zeleni protein se je izkazal kot toksičen za NRK celično lininjo. Razvili smo metodo merjenja intenzitete fluorescence ekstraktov NRK celic, ki so bile podvržene transfekciji. NRK celice transfektirane z vektorjem pEGFP-N_2 so izkazovale fluorescenco enakomerno razporejeno v citoplazmi in hkrati tudi zvečano kopičenje proteina v jedru. Kadar pa je GFP sklopljen z eksonom 2 Piruvat karboksilaze, je protein nakopičen v mitohondrijih. To lahko pripišemo lastnosti eksona 2, kateri nosi signal za vnos v mitohondrije. Iz primerjave intenzitete fluorescence med strukturami, katerih razlike izhajajo iz razlik na 5´ koncu informacijske RNA piruvatne karboksilaze, lahko sklepamo, da strukture proksimalnega promotorja zmanjšajo izražanje GFP. Razvili smo postopek za merjenje količine informacijske RNA za zeleni fluorescirajoči protein v celicah, ki so bile podvržene transfekciji.

Use of Green Fluorescent Protein in Transfected NRK Cells to Study Translation Rate from Different mRNA Isoforms of Pyruvate Carboxylase

S. Vozelj
Faculty of Pharmacy, Granada, Spain
Supervisor: A. Mlinarič
Faculty of Pharmacy, University of Ljubljana, Slovenia
Co-supervisor: A. Morales Vargas
Faculty of Pharmacy, Granada, Spain

Pyruvate carboxylase is a mitochondrial enzyme accomplishing central roles in a control of metabolism. In order to study the regulation of the expression of pyruvate carboxylase we constructed plasmids in which the expression of green fluorescent protein is controlled by pyruvate carboxylase rat gene promoters.
The six 5' ends of the different isoforms of the mRNA for pyruvate carboxylase, including the signal sequence to sort proteins to mitochondria, cloned in pEGFP-N_2 were used to study their influence on the expression of the fused protein. Permanent transfection of stable cell line from rat kidney failed. The synthesized protein from reporter gene is probable toxic for this cell line
The method to measure fluorescence intensity in extracts from transfected cells was optimized. The isoforms of mRNA drived from proximal promoter produce a diminution of green fluorescent protein expression. This fenomena can be ascribed to the instability of mRNA or to the blockade of translation.
A DNA probe for green fluorescent protein was designed in order to measure the transcription rate of green fluorescent protein in transfected cells.

Sinteze in pretvorbe metil (*Z*)-2-aroilamino-3-dimetilaminopropenoatov in poskusi kombinatorne sinteze na osnovi le-teh

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

Metil (*Z*)-2-benzoilamino-3-dimetilaminopropenoat je dobro znan reagent, ki se uspešno uporablja v sintezi različnih α-aminokislin in različnih heterocikličnih sistemov, ki v svoji strukturi vsebujejo α-aminokislinski fragment. V okviru našega dela smo želeli pripraviti nove reagente takšnega tipa, in sicer različne metil (*Z*)-2-aroilamino-3-dimetilaminopropenoate, pri katerih aroilni del predstavlja različno substituirani benzoilni fragment. V drugi polovici našega dela smo nove reagente preizkusili v kombinatorni kemiji v raztopini, relativno novem pristopu k organski sintezi. Cilj kombinatorne sinteze je bil študij poteka reakcije izmenjave dimetilaminske skupine metil (*Z*)-2-aroilamino-3-dimetilaminopropenoatov z različno substituiranimi fenilamini, ter določitev strukture tako dobljenih produktov. Ugotovili smo, da omenjeni tip reakcije pod določenimi pogoji v kombinatorni sintezi v raztopini zelo dobro poteka. Pri določanju strukture produktov [metil (*Z*)-3-arilamino-2-aroilaminopropenoati], pa smo potrdili (*Z*) konfiguracijo okoli C=C dvojne vezi, ki je bila na podobnih spojinah že določena.

Synthesis and Transformation of Methyl (*Z*)-2-aroylamino-3-dimethylaminopropenoates and their Potential Application in Combinatorial Chemistry

J. Wagger
Faculty of Pharmacy, University of Ljubljana, Slovenija
Supervisor: B. Stanovnik
Co-supervisor: J. Svete
Faculty of Chemistry and Chemical Tehnology, University of Ljubljana, Slovenia

Methyl (*Z*)-2-benzoylamino-3-dimethylaminopropenoate is a well-known reagent which has been already successfully used in the synthesis of various α-amino acids and various heterocyclic systems which have in their structure an α-amino acidic fragment. In our work, we have tried to prepare new reagents of such type, that is various methyl (*Z*)-2-aroylamino-3-dimethylaminopropenoates in which aroyl part represents variously substituted benzoyl fragment. In the second part of our work we have applied newly synthesized methyl (*Z*)-2-aroylamino-3-dimethylaminopropenoates in relatively new, and with such reagents never tried, approach toward chemical synthesis that is a solution phase combinatorial synthesis, where we performed substitution (addition-elimination) of dimethylamino group of prepared reagents with variously substituted phenylamines. We have also determined structures of products prepared by substitution reaction. We have found out that the performed type of reaction, under the determined conditions, runs very well. By structural determination of products [Methyl (*Z*)-3-arylamino-2-aroylaminopropenoates], the (*Z*) orientation around C=C double bond was confirmed.

Usklajevanje tehnologij za kemijsko sintezo učinkovin z evropskim regulatornim sistemom

R. Zajc
Krka, d. d., Novo mesto, Slovenija
Mentor: M. Japelj
Krka, d. d., Novo mesto, Slovenija
Somentor: M. Veber
Fakulteta za kemijo in kemijsko tehnologijo, Univerza v Ljubljani, Slovenija

Postopek pridobitve dovoljenja za promet z zdravilom v EU je odvisen od navodil in zahtev, ki se sprejemajo na nivoju EU in so implementirane v vsako izmed držav članic z nacionalnim zakonom in speljane v veljavo preko nacionalnih agencij. Namen postopka pridobitve dovoljenja za promet je predvsem ocena kakovosti, učinkovitosti in varnosti zdravila. Kvaliteta generičnega zdravila je dokazljiva z uporabo najprimernejših metod zbranih ali v evropski farmakopeji, ki je v EU zakonsko določena, ali v primeru zdravilne učinkovine, katere monografija se ne nahaja v evropski farmakopeji, izbor metod v skladu z določili ICH navodil. Osredotočili smo se na sintezo enalapril maleata, zdravilno učinkovino, za katero smo v Krki d. d. razvili lasten tehnološki postopek za njegovo sintezo in ga prenesli iz laboratorijskega v polindustrijsko in v industrijsko merilo. Pri tej sintezi smo obravnavali problematiko uporabe optimalnega izbora in regeneracije topil (metilen klorida, etil acetata in acetonitrila). Ugotavljali smo, katere so minimalne zahteve po kakovostih posameznih organskih topil (svežih in regeneriranih), v takšnih »kvalitetah«, da še ne vplivajo na končno, sicer natančno določeno »farmakopejsko kvaliteto« enalapril-maleata. V delu ugotavljamo tudi kako nujen je sistem obvladovanja sprememb, kajti vse spremembe, ki jih naredimo pri sami kemijski sintezi morajo biti usklajene s postopkom za obvladovanje sprememb, dobro proizvodno prakso in izpeljane v skladu z obstoječimi regulatornimi predpisi.

Harmonization of Technologies for Chemical Synthesis of Active Substances with the European Regulatory System

R. Zajc
Krka, d. d., Novo mesto, Slovenia
Supervisor: M. Japelj
Krka, d. d., Novo mesto, Slovenia
Co-supervisor: M. Veber
Faculty of Chemistry and Chemical Technology, University of Ljubljana, Slovenia

Registration of medicinal products within the EU is governed by the instructions and requirements reached at the EU level, introduced into each member country by their national laws and carried out by the national agencies. The main purpose of the registration procedure is assessment of the quality, efficacy and safety of medicinal products. Quality of a generic product can be proven by using the most appropriate methods collected either in the European Pharmacopoeia, which is governed by the law in the EU or, in the event of an active substance without a monograph in the European Pharmacopoeia, a selection of methods in compliance with the ICH directions. We have focused on the synthesis of enalapril maleate, an active substance for which Krka has developed its own technological procedure of synthesis. In the scope of this synthesis, we have dealt with the problems related to the use of the optimal choice and regeneration of solvents (methylene chloride, ethyl acetate and acetonitrile). We have tried to find out what are the minimal requirements for the quality of individual organic solvents (fresh and regenerated solvents) at which they still do not affect the final, precisely defined "pharmacopoeial quality" of enalapril maleate. The importance of the change control system has also been pointed out because all the changes made at the chemical synthesis must be consistent with the change control of the procedure, good manufacturing practice and carried out in accordance with the current regulatory rules.

Alge kot pokazatelji prikrite evtrofnosti reke Krke

A. Zorko
Biotehniška fakulteta, Univerza v Ljubljani, Slovenija
Mentor: M. J. Toman
Biotehniška fakulteta, Univerza v Ljubljani, Slovenija

V reki Krki so že pred leti opazili pojav "cvetenja" alg, kot posledica preobremenjenosti oz. evtrofikacije (Ev). V spodnjem počasi tekočem delu reke so glede temperature, svetlobe ter vsebnosti hranil ugodni pogoji za nastop Ev. Kot bioindikatorje Ev smo uporabili perifitonsko (Pr) in potamoplanktonsko (Pl) združbo, saj se Ev najprej odrazi na primarnih producentih. Od junija 2001 do maja 2002 smo vzorčili na 6 vzorčnih mestih. Namen dela je bil ugotoviti, ali prihaja do Ev v Krki ter najti vzroke zanjo. Ves čas smo merili fizikalne in kemijske parametre, kar smo združili s podatki o vplivu prispevnega področja. Združbo Pr in Pl smo kvalitativno analizirali in z Jaccardovim indeksom podobnosti ugotavljali podobnosti med združbama. V poletnem času je namnožena biomasa alg porabila hranila, povišali sta se koncentracija klorofila *a* in količina organskih snovi. Razmere za razvoj alg proste vodne mase v Krki so ugodne. Izvor Pl je lahko v Pr istega ali gorvodnega vzorčnega mesta. Prevladovale so diatomeje, poleti so jih zamenjale zelene alge.

Algae as Indicators of Latent Eutrophication of the Krka

A. Zorko
Biotehnical Faculty, University of Ljubljana, Slovenia
Supervisor: M. J. Toman
Biotehnical Faculty, University of Ljubljana, Slovenia

Algal bloom in Krka river (Slovenia) was observed some years ago, due to increased eutrophication (Eu). In lower slow-flowing reaches the conditions for Eu proces were favourable, regarding to temperature, light and nutrient countent. The direct result of Eu is an increase in productivity of periphyton (Pr) and potamoplankton (Pl) communities, therefore could serve as good bioindicators. We were sampling on 6 sites, from June 2001 to May 2002, including physical and chemical variables and pollution impact from catchment area. The purpuse of our work was to find out if the Eu is taking place in Krka river and point out the reasons for it. The samples were qualitatively evaluated. The Jaccard coefficient index was used to compare Pr and Pl assemblanges. During the summer algae used nutrients for intensive growth, that results in increased chlorophyll *a* concentration and increased amount of organic matter. The conditons for development of algae in water column were favourable. We also found out that many cells in the water column are simply sloughed material in transit from attached autotrophs. The Krka river was dominated by diatoms, while chlorophytes become more abundant in the summer period.

Določevanje pesticidov v zeliščih

V. Zupančič
Fakulteta za kemijo in kemijsko tehnologijo, Ljubljana, Slovenija
Mentorica: L. Zupančič-Kralj
Fakulteta za kemijo in kemijsko tehnologijo, Ljubljana, Slovenija

Pesticidi so sintetične kemikalije, ki jih uporabljamo za uničevanje škodljivcev. Zaradi nijihovega škodljivega učinka na zdravje ljudi zakonodaja postavlja visoke norme o maksimalni dovoljeni količini pesticidov v živilih.
V diplomskem delu sem skušal z eno metodo določiti nekaj predstavnikov različnih skupin pesticidov v zeliščih. Od vseh predpisanih pesticidov sem izbral dvanajst predstavnikov posameznih skupin, in sicer: alaklor, aldrin, dieldrin, endosulfan, heksaklorobenzen, heptaklor, lindan, piperonil butoksid, metil pentaklorofenilsulfid, diazinon, karbofenotion in malation. Za optimizacijo metode sem najprej določeval izbrane pesticide v vodi, potem v solati in nato v posušenih zeliščih. Ker so koncentracije pesticidov v realnih vzorcih ponavadi zelo nizke, sem se ukvarjal predvsem z ekstrakcijo na trdno fazo, ki je služila za koncentriranje in čiščenje ekstraktov. Za detekcijo sem uporabil plamensko-ionizacijski detektor. Izkoristki ekstrakcij pesticidov iz vode so znašali približno 90 % (v vodi jih na ta način lahko določimo še 2 mg/L), s solate pa približno 70 %. V izbranem koncentracijskem območju 8–50 mg/kg je odvisnost površine kromatografskih vrhov pesticidov od koncentracije linearna. V sveži solati lahko s to metodo določimo še 7 mg/kg posameznih pesticidov. Analizni postopek za določanje pesticidov v solati sem uporabil za določanje pesticidov v zeliščih. Izkazalo se je, da ekstrakti zelišč vsebujejo veliko spojin, ki pri kromatogafiranju povzročijo veliko kromatografskih vrhov. Z uporabo masnospektrometričnega detektorja sem večino interferenčnih vrhov identificiral. Za identifikacijo pesticidov v ekstraktih zeliščih sem zato uporabil selektivno detekcijo, in sicer masno spektrometrijo (snemanje kromatogramov pri izbranih karakterističnih ionih). Tako je spodnja meja določevanja pesticidov približno 1 mg pesticidov/kg posušenih zelišč.

Determination of Pesticides in Herbs

V. Zupančič
Faculty of Chemistry and Chemical Technology, University of Ljubljana, Slovenia
Supervisor: L. Zupančič-Kralj
Faculty of Chemistry and Chemical Technology, University of Ljubljana, Slovenia

Pesticides are synthetic chemicals used to destroy pests. Due to their negative influence on the health of people high norms about their maximum allowed quantity in provisions have been determined.
In my diploma work I used one method to determine some representatives of various groups of pesticides in herbs. Of all the prescribed pesticides I chose twelve representatives of various groups, i. e. alachlor, aldrine, dieldrine, endosulphan, hexachlorobenzene, heptachlor, lindane, piperonyl butoxide, methylpenta chlorophenilsulphide, diazinone, carbophenotion and malathion. To optimize the method I first determined the chosen pesticides in water, then in salad and finally in dried herbs. As the concentrations of pesticides are usually very low in real samples, I dealt mostly with the solid phase extraction which was used for concentration and purifying of the extracts. For the detection I used flame-ionization detector. The average yield of extraction of the pesticides in water was approximately 90 % (that way pesticides in water can be determined up to 2 mg/L), and in salad about 70 %. In the chosen concentration interval of 8–50mg/kg the dependence of chromatographic peaks area on concentration was linear. Using this method up to 7 mg/kg individual pesticides in fresh salad can be determined. To determine pesticides in hebs I used the same procedure as with pesticides in salad. It turned out that herb extracts contain many compounds which cause numerous chromatographic peaks. By the means of mass-spectrometric detector I managed to identify most of the interference peaks. For identification of the pesticides in herbs I used selective detection, namely mass spectrometry (selected ion chromatography). The limit of pesticide detection is approximately 1 mg of pesticides per kg of dried herbs.

Uporaba vodikovega peroksida in fluoriranih alkoholov za sintezo tetraoksanov z antimalarijsko aktivnostjo

K. Žmitek
Fakulteta za kemijo in kemijsko tehnologijo, Univerza v Ljubljani, Slovenija
Mentor: M. A. Zupan
Fakulteta za kemijo in kemijsko tehnologijo, Univerza v Ljubljani, Slovenija
Somentor: J. Iskra
Institut Jožef Stefan, Ljubljana, Slovenija

V priloženem delu smo optimizirali kislinsko katalizirano oksidativno ciklizacijo cikloheksanonskih derivatov v dispiro-1,2,4,5-tetraoksane, ki so nova skupina antimalarikov, aktivnih proti parazitom, odpornim na klasična protimalarijska zdravila (kininski derivati). Raziskali smo vpliv faktorjev, ki usmerjajo nastanek cikličnih peroksidov tetraoksanskega tipa in študirali vpliv različnih topil, kislin in metiltrioksorenija (MTO) kot katalizatorja na selektivnost reakcije za nastanek tetraoksanov. Z uporabo oksidacijskega sistema 0.1mol% MTO/1ekv. 30%H_2O_2/1ekv. HBF_4 v fluoriranem alkoholu (1,1,1-trifluoroetanol - TFE in 1,1,1,3,3,3-heksafluoro-2-propanol - HFIP) smo sintetizirali nove biološko pomembne tetraoksane z dobrim izkoristkom (46-86%) z direktno kislinsko katalizirano oksidativno ciklizacijo različnih 4-substituiranih cikloheksanonov (Me, Et, tert-Bu, CF_3, COOEt, Ph). Pri tem ima substituenta na mestu 4 cikloheksanonskega obroča velik vpliv na potek reakcije.

Synthesis of Tetraoxanes with Antimalarial Activity by Use of Hydrogen Peroxide in Fluorinated Alcohols

K. Žmitek
Faculty of Chemistry and Chemical Technology, University of Ljubljana, Slovenia
Supervisor: M. A. Zupan
Faculty of Chemistry and Chemical Technology, University of Ljubljana, Slovenia
Co-supervisor: J. Iskra
Jožef Stefan Institute, Ljubljana, Slovenia

In our work we have optimised acid-catalyzed oxidative cyclization of cyclohexanone derivatives to dispiro-1,2,4,5-tetraoxanes that are novel class of antimalarials active against chloroquine resistant parasites. Here we present our investigation on the factors that govern the formation of cyclic peroxides – tetraoxanes and we have studied the influence of different solvents, acids and methytrioxorhenium (MTO) as catalyst on the reaction selectivity for tetraoxane formation. The oxidative system 0.1mol% MTO/1eqv. 30%H_2O_2/1eqv.HBF_4 in fluorous alcohol (1,1,1-trifluoroethanol - TFE in 1,1,1,3,3,3-hexafluoro-2-propanol - HFIP) was found to be very promising for the synthesis of novel biologically important tetraoxanes with good yield (46-86%) by direct acid-catalyzed oxidative peroxidation of various 4-substituted cyclohexanones (Me, Et, tert-Bu, CF_3, COOEt, Ph). The conditions employed revieled that the substituent on position 4 of cyclohexanone ring had a big effect on the transformation.

C vitamin – kako ga obdržati čim več?

T. Brezovar, P. Hočevar, L. Kambič, K. Zorc
Gimnazija Novo mesto, Novo mesto, Slovenija
Mentorica: M. Kovač
Somentorica: M. Strmole
Gimnazija Novo mesto, Novo mesto, Slovenija

Namen naše raziskave je bil določiti vsebnost vitamina C v svežem soku rdeče paprike in pomaranče pod različnimi pogoji. Za omenjeni živili smo se odločile, ker je v njih vsebnost vitamina C razmeroma velika, sploh v primerjavi z ostalim sadjem in zelenjavo. Delo smo opravljale v šolskem laboratoriju. Sok paprike in pomaranče smo najprej iztisnile, ga nato prelile v razne posode (glineno, kovinsko, stekleno, plastično), in ga pustile na svetlobi oziroma v temi. Vzorce smo izpostavile različnim temperaturam in mu dodale različne dodatke. Skratka, sok smo izpostavile različnim dejavnikom. Nato smo merile vsebnost vitamina C, ki smo jo izvajale s pomočjo 2,6 – diklorfenolindofenola s titracijo. Meritve so pokazale zanimive rezultate: vrednost vitamina C je precej večja v rdeči papriki kot v pomaranči, spreminja pa se tudi glede na to, kje sok hranimo, pod kakšnimi pogoji in kako dolgo. Opisane meritve so predstavljale jedro naše naloge, medtem ko smo vzporedno opravile še meritve drugih značilnosti soka. Tako smo izmerile vsebnost suhe snovi, vode, v vodi topnih in tudi netopnih snovi. V nalogi je prikazan način dela, rezultati in izpeljani izračuni. Seveda je vse dopolnjeno še s potrebno teorijo, ki jo naši rezultati dokazujejo, na kratko pa je predstavljen tudi pomen vitamina C v vsakdanjem človekovem življenju.

Vitamin C – How to Keep it as Much as Possible?

T. Brezovar, P. Hočevar, L. Kambič, K. Zorc
Gimnazija Novo mesto, Novo mesto, Slovenia
Supervisor: M. Kovač
Co-supervisor: M. Strmole
Gimnazija Novo mesto, Novo mesto, Slovenia

In our research we wanted to determine the amount of vitamin C in the juice of a squeezed orange and in the juice of a squeezed red pepper. We decided for the red pepper and for the orange on the basis of the commonly – known fact that the amount of vitamin C in these vegetable and fruit is relatively high. Our research work was performed in the school laboratory. First, we separately squeezed the red pepper and the peeled orange. Then we poured both types of juice separately into different containers made from metal, glass and plastic. A certain amount of juice was put into the deep freeze, some of it were exposed to the light. We wanted to establish the effects of exposure to different outer factors on the content of vitamin C. The amount of vitamin C was determined by the titration using 2,6-dichloro phenolindophenole as indicator. The measurements showed interesting results. The amount of vitamin C in the juice of red pepper was much higher than that in the juice of the orange. The amount also changed according to the place of storage, according to the storing conditions and the duration of the storage. Along with the already mentioned research other measurements were carried out, showing other characteristics of the two types of juice. We determined the quantities of dry substance, of the water soluble components and of those that remained insoluble. In the research work the methods and tasks are presented together with the results and derived calculations. The results and calculations are upgraded with the theory. The importance of the presence of vitamin C in the daily nutriton is also emphasized.

Učinek različnih ekstraktov hrena na mikroorganizme in hren v prehrani

M. Franko, U. Potočar, M. Štimpfel
Kmetijska šola Grm Novo mesto, Slovenija
Mentorici: V. Zupančič, J. Goršin Fabjan
Kmetijska šola Grm Novo mesto, Slovenija

Korenina hrena vsebuje substanco sinigrin, ki je neke vrste tioglukozid. Pri mehanski obdelavi hrena (npr. nastrganje) sinigrin pod vplivom sproščenih encimov peroksidaze ali mirosinaze hidrolizira. Produkt te hidrolize je med drugim tudi spojina alil izotiocianat, ki je aktivna (inhibira rast) na določene mikroorganizme (tudi na nekatere patogene). Alil izotiocianat daje hrenu tudi značilno ostrino.
Namen te naloge je bil pripraviti različne ekstrakte hrena, jih kemijsko analizirati in jih med seboj primerjati na mikrobiološkem nivoju. Glede na znane podatke iz literature je bil naš cilj preveriti antiseptične učinke hrena oziroma njegovih ekstraktov, antibiotične učinke na Gram-pozitivne in na Gram-negativne bakterije ter učinke na rast gliv kvasovk. Zanimalo nas je, ali ostrina hrena vpliva na te učinke, torej, ali je glavna aktivna substanca v hrenu tista, ki mu daje značilno ostrino.
Pri našem delu smo uporabljali različne kemijske metode (ekstrakcija, destilacija z vodno paro, kromatografija (TLC)), spektroskopske metode analize (IR), mikrobiološke tehnike (nacepljanje kultur na gojišča, antibiogram - metoda za določanje učinkovitosti antiseptikov in razkužil) in se naučili pripravljati različne jedi. Hren je užitna rastlina, zato smo raziskali tudi njegov pomen v prehrani. Zbrali smo različne recepte za pripravo jedi, jih nekaj (15) tudi pripravili in degustirali.

The Effect of Different Horseradish Extracts on Microorganisms and Horseradish in Nutrition

M. Franko, U. Potočar, M. Štimpfel
Agricultural School Grm Novo mesto, Slovenia
Supervisors: V. Zupančič, J. Goršin Fabjan
Agricultural School Grm Novo mesto, Slovenia

Synigrin, a thioglucoside of cruciferae plants, such as horseradish, may be enzymatically hydrolized (enzymes peroxidase or myrosinase), to yield up to different substances when horseradish is mechanically disrupted (e.g. scraping). One of those substances is allyl isothiocyanate, which inhibits many types of micro-organisms, some of which are pathogenic. Allyl isothiocianate is responsible for the pungent flavour of horseradish.
The aim of the project was to prepare different horseradish extracts, to analyse their chemical structure and to compare them on the microbiological level. Our objective was to check antiseptical effects of horseradish and its extracts, antibiotic effects on Gram positive and Gram negative bacteria, as well as the growth effects of yeasts, according to the known data from the literature. We wanted to find out if the pungent flavour of horseradish is due to the main active substance.
During the project different chemical methods (extraction, water vapor destillation, chromatography (TLC), spectroscopical analytical methods (IR), and microbiological techniques (different antibiograms) were used.
Horseradish is an edible plant and therefore we also investigated its importance in the nutrition. We gathered various recipes for preparing different dishes which contain horseradish. In addition we have also prepared some of them (15) and tasted them as well.

Testiranje Hydronic vitalizatorja v tehnologiji priprave procesne vode

I. Kadič, I. Veselič, J. Zadnik
Gimnazija Novo mesto, Slovenija
Mentorice: S. Kermc, H. Kotnik
Krka, d. d., Novo mesto, Slovenija
B. Bajc Šerovič
Gimnazija Novo mesto, Slovenija

V današnjem svetu je čedalje več potreb po čisti pitni vodi. Število prebivalstva se veča, prav tako je več industrije, ki za svojo proizvodnjo porabi nepredstavljive količine pitne vode. Viri čiste pitne vode so omejeni in ne zagotavljajo dovolj vode niti za potrebe ljudi. Prav zato so v industriji uveljavljeni postopki za pridelavo čiste neoporečne vode iz razpoložljivih, največkrat onesnaženih virov. Ta tehnologija je danes že zelo razvita, vendar pri svojem delu potrebuje veliko energije. Ta problem je vzpodbudil nekatere raziskovalce, da so začeli stremeti po novih načinih za prečiščevanje vode. Ena izmed takšnih inovacij je tudi vitalizator vode Hydronic. V naši raziskovalni nalogi smo preizkusili vitalizator Hydronic, da bi ovrednotili njegov vpliv na vodo, ki se pretaka skozenj. Na tržišču se je pojavil pred tremi leti skupaj s kozarci za vitalizacijo vode. Izum je poleg številnih drugih priznanj leta 2000 na sejmu v Pittsburghu v ZDA prejel veliko zlato medaljo in posebno priznanje za najboljši izum leta na področju ekologije. Prejetje odličja je zbudilo zanimanje stroke in od takrat je bilo narejenih kar nekaj raziskav o vplivu Hydronica na vodo. Raziskovalci so preučevali predvsem vidne spremembe na vodi ali živalih, ki so to vodo zaužile. V Krki, tovarni zdravil, d. d., pa jih je zanimala predvsem sposobnost Hydronica kot prečiščevalne predpriprave reke Krke za nadaljnje pridobivanje procesne vode. V primeru, da bi imel sposobnost znižanja določenih parametrov, bi omogočil velik prihranek prostora, energije ter kontinuirano kvaliteto.

Testing the Hydronic Vitalizer in Process Water Preparation

I. Kadič, I. Veselič, J. Zadnik
Gimnazija Novo mesto, Slovenia
Supervisors: S. Kermc, H. Kotnik
Krka, d. d., Novo mesto, Slovenia
B. Bajc Šerovič
Gimnazija Novo mesto, Slovenia

Nowadays, world clean drinking water is more and more in demand. Growing population and greater demands of industry increase the need for the potable water. The sources of drinking water are restricted and they do not guarantee sufficient quantities even for human needs. In the past few years, great efforts were put into the methods for extraction of clean, potable water from available, mostly polluted sources. The modern technology has been already very improved. This problem has stimulated several researchers to look for new ways of water purification. A water vitalizer Hydronic is one of the results of such efforts. In our research work we have tested the vitalizer Hydronic to determine its impact on the quality of water. It was put on the market together with drinkingcups for water vitalization three years ago. Beside numerous other awards, this invention won the big gold medal and special award for the greatest ecological invention of the year at Pittsburgh fair (USA) in the year 2000. This prominent prize has awoken the interest of profession and a lot of research has been done to prove its efficacy. Researchers have mostly studied visible changes of the water or animals that have consumed this water. In Krka, pharmaceutical company, there was an interest in ability of Hydronic to purify water which can be used for acquirement of process water from Krka river. In the case that Hydronic would lower some quality parameters, it would enable great savings of manufacturing space and energy as well as assure continuous quality.

Marketinška raziskava dietetičnih izdelkov

T. Kočevar, R. Kalčič
Gimnazija Novo mesto, Slovenija
Mentorica: T. Durmič
Gimnazija Novo mesto, Slovenija
Somentorica: K. Kikelj
Krka, d. d., Novo mesto, Slovenija

Dietetične izdelke lahko drugače imenujemo tudi prehranski dodatki. To so razni vitamini, minerali in oligoelementi, ki jih dodajamo našemu telesu z željo po boljšem počutju, boljšemu delovanju organizma, izboljšanju obrambnega mehanizma. Sestavine zdrave prehrane so vitamini in minerali.
Najino nalogo sva razdelili na teoretični in eksperimentalni del. V prvem sva se osredotočili na vitamine in minerale, na njihovo uporabo, delovanje, stranske učinke. Najpomembnejše vitamine sva opisali in navedli tudi njihovo vlogo. Prav tako sva opisali vlogo in delovanje najpomembnejših mineralov. Opisane so tudi potrebe dojenčkov, otrok in nosečnic, varnosti pri uporabi. V najino nalogo pa sva vključili tudi regulativo na področju prehranskih dopolnil - regulativo EU, nacionalne/lokalne zakonodaje, pa tudi zakonodajo na področju prehranskih dopolnil v Sloveniji. Eksperimentalni del vključuje anketo, s katero sva dobili večji vpogled na splošno znanje in osveščenost ljudi: kje kupujejo dietetične izdelke, kaj je za njih pomembno pri nakupu, katere izdelke so že uporabljali, ali je pri nakupu pomembna embalaža, zakaj se odločajo za nakup dietetičnih izdelkov. Dobili sva podatke o nakupnih navadah, mnenju glede razpoznavnega znaka za Krkine izdelke, osveščenosti glede vloge določenih vitaminov. Rezultate sva prikazali v obliki grafov in s tem dobili še boljšo vizualno predstavo.

Marketing Research of Dietetic Products

T. Kočevar, R. Kalčič
Gimnazija Novo mesto, Slovenija
Supervisior: T. Durmič
Gimnazija Novo mesto, Slovenia
Co-supervisor: K. Kikelj
Krka, d. d., Novo mesto, Slovenia

Nutritional additives is another name applied to dietetic products. These are various vitamins, minerals and oligoelements, which we consume to achieve a better state of health, a better performance of our organism and to strengthen our defense system. Vitamins and minerals constitute the vital components of healthy diet.
Our research project is divided into two parts, a theoretical and an experimental one. The first part focuses on the role of the most important vitamins and minerals, their use, mode of action and their side effects. In addition, the project describes the vitamin/mineral needs of infants, children and pregnant women. It also indicates safety during the use of these dietetic products. Furthermore, we included some regulation data from the field of the nutritional additives – the EU regulation, national/local legislation, as well as legislation in the field of the nutritional additives in Slovenia. The experimental part was carried out in the form of the survey with which we got a better insight into people's general knowledge and awareness. The main questions asked were where they buy dietetic products, what they find important when purchasing these products, which products have they already used, whether they find packaging of the product important and why they decide to buy dietetic products. The results thus obtained showed the buying habits, opinions on the recognition of the logo of Krka's products and awareness of the role of particular vitamin. To attain better visual representation, some of the results are presented in the form of a diagram.

Optimizacija prekristalizacije oksitetraciklin dihidrata v vodnem mediju

M. Repše, D. Bele, M. Luzar
Gimnazija Novo mesto, Slovenija
Mentor: J. Tihi
Krka, d. d., Novo mesto, Slovenija

Antibiotik oksitetraciklin dihidrat spada v skupino tetraciklinskih antibiotikov, odkritih v zgodnjih 50. letih. Pridobivamo ga z aerobno fermentacijo *Streptomyces rimosus*.
Antibiotik OTC je spojina, ki je zelo dobro topna v kislinah in lugih. To njegovo lastnost s pridom uporabljamo pri izolaciji iz fermentacijske brozge, kjer najprej pri nizkem pH-ju ekstrahiramo antibiotik iz micelija in ga nato oborimo s spremembo pH pri njegovi izoelektrični točki. Med samo fermentacijo mikroorganizem proizvaja tudi sorodne spojine, in sicer tetraciklin in ADOTC. Med samo izolacijo pa lahko nastane tudi 4-epi oksitetraciklin.
Namen naše naloge je bil študij in optimizacija dodatnega čiščenja tako pridobljenega antibiotika.
Ugotovili smo, da je nujna uporaba reagenta BN, kot sama izvedba prekristalizacije pri povišani temperaturi. Pri študiju prekristalizacije smo prišli do zaključka, da se lahko izognemo uporabi reagenta Q-50. Poleg izvedbe same prekristalizacije je pomemben vpliv kvalitete izhodnega oksitetraciklin dihidrata.

Optimization of Recrystallization of Oxytetracycline Dihydrate in Water Media

M. Repše, D. Bele, M. Luzar
Gimnazija Novo mesto, Slovenija
Supervisor: J. Tihi
Krka, d. d., Novo mesto, Slovenia

Antibiotic OTC·2H_2O belongs to the group of tetracycline antibiotics which were discovered in the early fifties. It is obtained with aerobic fermentation of *Streptomyces rimosus*. Antibiotic OTC is a compound which has good solubility in acids and bases. This property could be used for isolation from fermentation broth where it is firstly extracted the antibiotic from mycelium at low pH and then precipitated with the variation of pH at its isoelectrical point. During the fermentation micro organism produces related compounds as well (tetracycline, ADOTC). During isolation 4-epi OTC can be produced.
Our intention was study of purification of so obtained antibiotic. It was found out that use of reagent BN is urgent as well as recrystallization itself at increased temperature. In study of recrystallization we came to a conclusion that the use of reagent Q-50 can be avoided. Besides recrystallization impact of quality of starting oxytetracycline dehydrate is significant.

Seznam avtorjev / Author Index

Seznam mentorjev / Supervisor Index

Uredniški odbor

Natalija Vitezić, glavna urednica
Miha Japelj
Mateja Vukšinič Kozoglav

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