KRKA

34. Krkine nagrade

34th Krka Prizes

Novo mesto, Slovenija, 28.-29. oktober 2004

14. mednarodni simpozij
14th International Symposium

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Častni odbor 34. 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

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Miha Japelj
Jože Drinovec
Natalija Vitezić
Stanislav Zalar
Alenka Kralj-Pučko
Ivan Radež
Elvira Medved
Dušan Dular
Aleš Hvala
Jože Gnidovec
Irena Čarman
Damjan Možina

Znanstveni odbor 14. mednarodnega simpozija

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Natalija Vitezić
Janko Kos
Jože Drinovec
Franc Vrečer

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Vsebina

34. Krkine nagrade / Krka Prizes

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

34. Krkine nagrade
34th Krka Prizes

34

Znanje zmaguje – Sciencia vincens

Letošnje leto bo marsikomu ostalo v spominu kot prelomno. V Evropi je prišlo do pomembnih sprememb. Samo ena med njimi je širitev Evropske Zveze. Spremembe se vrstijo z vrtoglavo naglico, tudi na področjih izobraževanja in raziskav. V življenje prehaja Bolonjska deklaracija, katere namen je odpreti in z novim vetrom pognati svežo kri po žilah izobraževalnih ustanov. Postavljajo se novi koncepti evropskemu raziskovalnemu prostoru, ki naj bi vzpodbudili širšo sodelovanje na kontinentu. Ambicije evropskih držav so velike, izzivi prav tako.
Ob teh velikih, morda zgodovinskih premikih, pa je slej ko prej jasno, da napredek temelji na vsakem posamezniku, njegovem delu, ustvarjalnosti in motiviranosti. Pomemben del našega skupnega uspeha ste tudi Krkini nagrajenci in mentorji. Z vsakim dosežkom posebej ste zrasli sami, s tem pa tudi prispevali k rasti in razvoju vseh nas. V Krki to pot dobro poznamo in cenimo, saj smo na tak način zrasli in po 50 letih dosegli odličnost, ki nas obvezuje za naprej. Veseli bomo, če se bodo naše poti srečale tudi v prihodnosti, danes pa se veselimo vaših dosežkov!

Knowledge Wins

This year will be remembered as a turning point. Europe has undergone significant changes, expansion of the European Union being just one of them. Changes are taking place at enormous speed also in the field of education and research. The Bologna Declaration, which is aimed at opening and revitalizing educational institutions, is becoming a reality. New concepts are being set to the European research area in order to stimulate widespread co-operation on the Continent. European countries have great ambitions but they are also facing great challenges.
The current changes may be great and even historical, but it is nevertheless clear that progress is always based on individuals, their work, creativity and motivation. Krka's Prize Winners and mentors constitute an important part of our joint success. Each individual achievement has contributed to your personal growth and also to the growth and the development of all of us. We, at Krka, appreciate and are well familiar with this path, which we have followed for 50 years to reach the present excellence, which we find binding also for the future.
We will be glad if our paths join in the future but today, we are sharing delight at your achievements.

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 2004 / Krka Prize Winners 2004

Št.	Nagrajenci	Status	Mentor, Somentor(ji)
1933	MATEJA ANTONČIČ	študentka farmacije	Mojca KRŽAN
1934	BOJANA BAŠELJ	dijakinja	Tatjana DURMIČ Tatjana HARLANDER
1935	NINA BEDNARŠEK	študentka Biotehnične fakultete	Romana MARINŠEK LOGAR Ester HEATH
1936	EVA BERCE	študentka farmacije	Marija BOGATAJ
1937	MIRJANA BERITIČ	dijakinja	Aleš GASPARIČ
1938	POLONCA BESAL	študentka ekonomije	Neva MAHER
1939	JURE BEZENŠEK	dijak	Katarina MERLIN Marinka KOVAČ
1940	AJDA BIČEK	študentka Biotehnične fakultete	Mojca NARAT Dušan BENČINA
1941	PETRA BOHANEC	študentka kemije	Katja BRESKVAR Vita DOLŽAN
1942	PETER CIMERMANČIČ	dijak	Katarina MERLIN Marinka KOVAČ
1943	TINA CIRMAN	doktorandka	Boris TURK Vito TURK
1944	MARJETKA CIZELJ	študentka kemije	Andreja ZUPANČIČ VALANT
1945	EMILIJA CVETKOVSKA	podiplomska študentka medicine	Ilija DZONOV
1946	IVANA ČERNE	dijakinja	Janja GORŠIN FABJAN Mojca GOLOB
1947	KATJA ČERNE	študentka medicine	Irena KEBER Mitja LAINŠČAK
1948	KRISTINA DAMJANOVIČ	dijakinja	Tatjana DURMIČ Tatjana HARLANDER
1949	MAJA DOBRAVC	študentka farmacije	Vojko KMETEC Mateja MERSLAVIČ
1950	NATAŠA DOLANC	študentka Biotehnične fakultete	Sonja SMOLE MOŽINA
1951	JERNEJA FARKAŠ	študentka medicine	Irena KEBER Mitja LAINŠČAK
1952	VESNA FLIS	študentka kemije	Tine KOLOINI Ivan RADEŽ
1953	BRUNA FORTUNA	študentka farmacije	Franc VREČER
1954	ROK FRLAN	študent farmacije	Danijel KIKELJ
1955	LUCIJA GALIČ	dijakinja	Janja GORŠIN FABJAN Mojca GOLOB
1956	BARBARA GERIČ	doktorandka	Miklavž GRABNAR Maja RUPNIK
1957	MOJCA GORENC	dijakinja	Marinka KOVAČ
1958	CENE GOSTINČAR	študent Biotehnične fakultete	Marina TURK Nina GUNDE-CIMERMAN
1959	URŠKA GRAČNER	dijakinja	Aleš GASPARIČ
1960	UROŠ GROŠELJ	doktorand	Jurij SVETE
1961	DANICA GRUŠOVNIK	študentka kemije	Alenka MAJCEN LE MARECHAL Darinka BRODNJAK VONČINA
1962	BOŠTJAN GUŠTIN	študent farmacije	Albin KRISTL
1963	MATJAŽ HOMAN	podiplomski študent medicine	Mario POLJAK
1964	MAGDA HUDOKLIN	podiplomska študentka ekonomije	Boris SNOJ

1965	JERNEJ HVALA	študent kemije	Matija STRLIČ Irena KRALJ-CIGIĆ
1966	MIRAN HVALEC	podiplomski študent kemije	Peter GLAVIČ Andreja GORŠEK
1967	URŠKA JAMNIKAR	študentka kemije	Darja BARLIČ-MAGANJA Marko DOLINAR
1968	PETRA JANC	dijakinja	Marinka KOVAČ
1969	DUŠAN JANEŽIČ	študent kemije	Janez PETEK Peter GLAVIČ
1970	JANJA JANEŽIČ	dijakinja	Janja GORŠIN FABJAN Mojca GOLOB
1971	NEJC JELEN	študent kemije	Damjana ROZMAN Tadeja REŽEN
1972	ŠPELA JENKO-BRINOVEC	doktorandka	Peter RASPOR
1973	BARBARA JERAJ	dijakinja	Irena KURAJIĆ Katja SEME
1974	NEJC KIC	dijak	Irena KURAJIĆ Katja SEME
1975	PETRA KOCBEK	podiplomska študentka farmacije	Julijana KRISTL
1976	JANEZ KONC	študent farmacije	Jure STOJAN
1977	BRANKA KOS	dijakinja	Aleš GASPARIČ
1978	NATAŠA KOS	študentka organizacije dela	Marko FERJAN Alenka KRALJ PUČKO
1979	MATEJA KOŠIČEK	podiplomska študentka ekonomije	Aleš GROZNIK
1980	KATERINA KOVAČEVIČ	študentka veterine	Gregor MAJDIČ
1981	ALENKA KRAJNC	študentka farmacije	Metka FILIPIČ Stanislav GOBEC
1982	KRIŠTOF KRANJC	doktorand	Marijan KOČEVAR
1983	BARBARA KUMELJ	študentka kemije	Janvit GOLOB Rok ZUPET
1984	PETER KUNEJ	podiplomski študent ekonomije	Danijel PUČKO
1985	MARKO LAMPRET	podiplomski študent strojništva	Iztok GOLOBIČ
1986	AJDA LAPORNIK	študentka Biotehnične fakultete	Tea LANIŠNIK-RIŽNER
1987	MATEJA LENARČIČ	podiplomska študentka ekonomije	Matej LAHOVNIK
1988	HELENA LENASI	doktorandka	Marin ŠTRUCL Metka BUDIHNA Ingrid FLEMING Rudi BUSSE
1989	VALENTINA LENASI	doktorandka	Peter DOVČ
1990	JAN LESKOVAR	študent farmacije	Vesna KROŠELJ Franc VREČER
1991	MARTINA MAKŠE	študentka kemije	Barbara MODEC
1992	BRONJA MANČEK	podiplomska študentka farmacije	Andrej UMEK Samo KREFT
1993	LUCIJA MANDELJ	študentka kemije	Lucija ZUPANČIČ-KRALJ
1994	DARJA MIHELČIČ	podiplomska študentka veterine	Marinka DROBNIČ-KOŠOROK
1995	URŠKA MOČNIK BONČINA	podiplomska študenka medicine dela	Metoda DODIČ FIKFAK Eva STERGAR
1996	DARJA MOHORČIČ	dijakinja	Tatjana DURMIČ Tatjana HARLANDER
1997	MARKO MUHIČ	dijak	Stanislava FLORIJANČIČ Marjeta REDEK
1998	ANDREJA NOVAK	podiplomska študentka komunikologije	Dejan VERČIČ Samo KROPIVNIK
1999	POLONA NOVAK	študentka farmacije	Jože DRINOVEC Aleš MRHAR
2000	IRENE NUZZO	doktorandka	Saverio FLORIO
2001	PETRA OBREZA	študentka farmacije	Mirjana GAŠPERLIN
2002	VERONIKA OKRŠLAR	doktorandka	Jana ŽEL

2003	METKA PAČNIK	študentka Biotehnične fakultete	Lovro STANOVNIK
2004	MUZAFERA PALJEVAC	študentka kemije	Maja HABULIN
			Željko KNEZ
2005	JELENA PAROJČIĆ	doktorandka	Zorica ĐURIĆ
2006	MATEJ PAVLI	študent farmacije	Natalija ZAJC
2007	MARGARETA PAVLIČ	študentka ekonomije	Rudolf ROZMAN
			Evridika MOROSINI BERUS
2008	ANDREJ PERDIH	študent farmacije	Tomaž ŠOLMAJER
			Marija SOLLNER DOLENC
2009	ANITA PESTOTNIK	študentka farmacije	Galina PUNGERČIČ
2010	TONI PETAN	doktorand	Jože PUNGERČAR
2011	ŽIVA PIPAN	študentka Biotehnične fakultete	Gregor SERŠA
			Maja ČEMAŽAR
2012	SAMO PIRC	doktorand	Branko STANOVNIK
2013	NEŽA PIŠEK	študentka farmacije	Mojca LUNDER
			Samo KREFT
2014	BARBARA PIŠKUR	študentka Biotehnične fakultete	Peter RASPOR
2015	ALEŠ PREMZL	doktorand	Janko KOS
2016	IRENA PULKO	študentka kemije	Peter KRAJNC
2017	TOMAŽ RAVNIKAR	študent farmacije	Petra SLANC
			Borut ŠTRUKELJ
2018	NADA RIBNIKAR	študentka farmacije	Borut BOŽIČ
			Saša ČUČNIK
2019	NINA RUSTJA	študentka sociologije	Marjan SVETLIČIČ
2020	ALLA JURJEVNA SAVCHENKO	podiplomska študentka medicine	Galina Vladislavovna RAMENSKAYA
2021	BARBARA SENEKOVIČ	študentka turizma	Marlena SKVARČA
2022	ANDREJA SENICA	študentka farmacije	Iztok GRABNAR
2023	MARKO SIMONIČ	dijak	Stanislava FLORIJANČIČ
			Marjeta REDEK
2024	MARIA STYCZYŃSKA	doktorandka	Maria BARCIKOWSKA
2025	ANJA ŠALEHAR	študentka farmacije	Borut ŠTRUKELJ
			Jerica SABOTIČ
2026	MARIO ŠIMIĆ	študent kemije	Jurij LAH
2027	DIANA ŠINKOVEC	dijakinja	Marinka KOVAČ
2028	METKA ŠIRCELJ	študentka farmacije	Janja MARC
2029	ANTON ŠKOF	podiplomski študent elektrotehnike	Boštjan LIKAR
2030	URŠKA ŠKOF	študentka farmacije	Marija SOLLNER DOLENC
2031	TINA ŠMUC	študentka kemije	Franc GUBENŠEK
2032	MATEJA ŠTEMPELJ	doktorandka	Ilonka FERJAN
2033	URBAN ŠVAJGER	študent farmacije	Stanislav GOBEC
2034	NATAŠA TANŠEK	študentka farmacije	Martina FINK
			Damjana ROZMAN
2035	ANDRIJANA TIVADAR	doktorandka	Stane SRČIČ
2036	ZOJA TRENZ	študentka organizacije dela	Vladislav RAJKOVIČ
2037	(TRŠAN) ŽAGAR TINA	študentka Biotehnične fakultete	Vita DOLŽAN
2038	VIKTOR URŠIČ	študent Biotehnične fakultete	Nina GUNDE-CIMERMAN
2039	TOMAŽ VAUPOTIČ	študent kemije	Ana PLEMENITAŠ
2040	ANJA VENTURINI	študentka kemije	Vladka ČURIN ŠERBEC
			Katrina PRETNAR HARTMAN
2041	JANA VERBIČ	študentka farmacije	Saša BAUMGARTNER
2042	ALJA VIDETIČ	študentka kemije	Radovan KOMEL
2043	TANJA VNUČEC	študentka kemije	Darinka BRODNJAK-VONČINA
			Aljana PETEK
2044	MATJAŽ VOGELSANG	študent kemije	Radovan KOMEL
			Saša COMINO
2045	KATJA VRABL	študentka farmacije	Klementina FON TACER
			Damjana ROZMAN
2046	IRENA VRTARIČ	študentka ekonomije	Andrej HARTMAN

2047	NATALIJA ZAJC	doktorandka	Odon PLANINŠEK
2048	JERNEJ ZIDAR	študent kemije	Peter BUKOVEC
2049	ALENKA ZUPANČIČ	študentka kemije	Andrej JAMNIK Marija BEŠTER ROGAČ
2050	JANEZ ZUPANČIČ	podiplomski študent kemije	Viktor GRILC Marjo MERSLAVIČ
2051	MAJA ŽLOGAR	podiplomska študentka ekonomije	Vesna ŽABKAR
2052	TANJA ŽUPEC	študentka kemije	Marija BEŠTER ROGAČ Darja RUDAN TASIČ

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14. mednarodni simpozij in 34. Krkine nagrade

Krkine nagrade postajajo že kar slovenski simbol spodbujanja mladih študentov in dijakov k poglobljenemu in vsestranskemu ustvarjalnemu delu; prednostno na tistih področjih sodobne znanosti, razvoja, raziskav in strokovnih vrhunskih specializacij, ki so ključnega pomena za razvoj moderne svetovne farmacevtsko-kemijske industrije. V teh 34 letih zelo uspešnega delovanja in uveljavljanja Krkinih nagrad smo (bomo) podelili našo nagrado že 2.052 nagrajencem.
Krkine nagrade že 14 let povezujemo z mednarodnimi simpoziji, na katerih predstavljajo izbrane raziskovalne in znanstvene dosežke vabljeni plenarni predavatelji iz številnih držav. Pri naši simpozijih posvečamo tudi posebno pozornost predstavitvam raziskovalnih dosežkov naših Krkinih nagrajencev. Mladi doktorji znanosti predstavljajo svoja doktorska dela s krajšimi predavanji, drugi naši nagrajenci pa predstavljajo svoje raziskave v Poster sekciji.
Do sedaj smo v Krko povabili že nad 100 uveljavljenih vrhunskih univerzitetnih profesorjev, akademikov in strokovnjakov (med drugim tudi Nobelovega nagrajenca) iz 25 držav Evrope in ZDA in Slovenije.

Na našem letošnjem simpoziju bodo sodelovali priznani profesorji in uveljavljeni strokovnjaki iz šestih držav: iz Rusije, Poljske, Nizozemske, Romunije, Hrvaške in Slovenije.

Na 14. mednarodnem simpoziju, ki ga bo s svečanim nagovorom odprl
predsednik Slovenske akademije znanosti in umetnosti, akademik prof. dr. Boštjan Žekš

bodo sodelovali naslednji vabljeni plenarni predavatelji:

Prof. dr. Jan van der Greef iz Nizozemske
Prof. dr. Jan van der Greef je znanstveni direktor v firmi TNO za sistemsko biologijo. Bil je direktor firme TNO Pharma, ki je postala vodilna organizacija, za izvajanje ekspertiz, modernih inovacij in raziskav za mednarodno farmacevtsko industrijo, tudi s pomočjo odkrivanja in razvoja zdravil. Poseben poudarek daje firma razvoju nove instrumentalne tehnike, ki je v sinergiji z biološkimi in biomediciskimi ekspertizami.
Prof. dr. Jan van der Greef je profesor analitike v bioznanosti na Univerzi v Leidnu in deluje v sklopu Leiden-Amsterdamskega Centra za raziskave zdravil (LACDR).
Danes se pri svojih raziskavah posveča razvoju sistemske biologije. Svoje raziskave je razširil na separacijske metode (LC, CE, CEC) v kombinacijah z masno spektrometrijo in kemometrijo na področju analitike v bioznanosti. Za svoje pomembne dosežke na področju analize zdravil je prejel nagrado belgijskega Društva za farmacevtske znanosti v letu 1998. Izbran je bil tudi za častnega doktorja na Univerzi v Gentu v letu 2000.

Prof. dr. Liviu Matcău iz Romunije
Prof. dr. Liviu Matcău je pomemben in uveljavljen nevrolog. Je profesorski vodja Nevrološke klinike okrožne bolnice in pomemben univerzitetni profesor na Univerzi za medicino in farmacijo »Victor Babeş« v Temišvarju.
Prof. Matcău je tudi predsednik fundacije za multiplo sklerozo in član fundacije za Alzheimerjevo demenco.
Svoje znanstveno in raziskovalno delo usmerja na nevroanatomijo, nevrološko okrevanje in nevrološko diagnostiko. Iz teh področij je objavil precej strokovnih knjig.
Prof. Matcău je član romunske akademije in član romunskega nevrološkega društva, član medicinskega združenja v Temišvarju in romunskega medicinskega kolegija.

Dr. Galina Vladislavovna Ramenskaya iz Rusije
Dr. Galina Ramenskaya je vodja Oddelka za klinične in farmakološke ekspertize za zdravila na ministrstvu za zdravje v Moskvi.

Je doktorica farmacevtskih znanosti in pomembna strokovnjakinja za področja farmakologije, klinične farmakologije in farmacevtske kemije. Svoje strokovno delo in aktivnost usmerja na področja predkliničnih in kliničnih preizkušanj zdravil, na raziskave biološke ekvivalence in na analizo učinkovin v zdravilih in v bioloških tekočinah.
Svojo znanstveno raziskovalno dejavnost usmerja na farmakokinetične in faramakogenetske študije metabolizma zdravil. Iz teh področij raziskav je objavila precej pomembnih publikacij.

Prof. dr. Maria Barcikowska iz Poljske
Prof. dr. Maria Barcikowska je vodja Oddelka za nevrodegerativne bolezni CNS in vodja Nevrološkega Oddelka na Medicinskem raziskovalnem centru pri poljski akademiji znanosti.
Prof. Barcikowska je uveljavljena poljska strokovnjakinja za nevrologijo in nevropatologijo. Svoje raziskovalno delo je osredotočila na klinične in nevropatološke pojave pri Alzheimerjevi bolezni in sorodnih bolezenskih motnjah. Rezultate svojih raziskav uspešno objavlja v številnih mednarodnih in domačih revijah.

Izredni profesor dr. Davorin Štimac iz Hrvaške
Dr. Davorin Štimac je vodja oddelka za gastroenterologijo v Kliničnem bolnišničnem centru na Reki. Je izredni profesor za interno medicino na Univerzi v Reki.
Dr. Štimac je pomemben hrvaški gastroenterolog. Svoj magistrski študij je opravil na področju farmakologije in disertacijo na področju eksperimentalne hepatologije.
Strokovno se je usposabljal v Italiji in Veliki Britaniji. Za svoje raziskovalne dosežke je prejel nekaj državnih in mednarodnih nagrad. Dr. Štimac je član številnih mednarodnih gastroenteroloških in hepatoloških združenj.
V mednarodnih revijah je objavil precej publikacij.

Doc. dr. Blanka Kores Plesničar iz Slovenije
Dr. Kores Plesničarjeva je docentka za psihiatrijo na univerzi v Ljubljani in vodja Oddelka za psihiatrijo v bolnici Maribor.
Je pomembna slovenska psihiatrinja. Svoje pedagoško in raziskovalno dejavnost usmerja na področja psihiatrije, psihoterapije, psihofarmakologije in klinične farmakologije.
Doc. dr. Blanka Kores-Plesničar je članica Psihofarmakološkega centra na Oddelku za psihiatrijo, predsednica komisije za zdravila na Univerzitetni psihiatrični kliniki v Ljubljani, članica skupine za klinične raziskave in predstavnica Slovenije v evropskih združenjih EMEA za učinkovitost in UEMS (evropskega medicinskega združenja za psihiatrijo). Je članica številnih slovenskih medicinskih združenj.

Svoja doktorska dela bo predstavilo s krajšimi predavanji tudi 13 Krkinih nagrajencev, novih doktorjev znanosti.
V posebni »Poster sekciji« pa bodo vsi preostali Krkini nagrajenci predstavili raziskovalne dosežke iz svojega diplomskega in magistrskega študija v obliki posterjev. Svoje raziskovalne dosežke bodo na »Poster sekciji« prikazali tudi najmlajši Krkini nagrajenci, dijaki iz dolenjskih srednjih šol.

Vsem vabljenim plenarnim predavateljem, mentorjem in 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
14. mednarodnega simpozija
prof. dr. Miha Japelj

14th International Symposium and 34th Krka Prizes

Krka Prizes have already become the Slovenian symbol of motivating young students to engage in extensive and universal creative work, primarily in the fields of modern science, development, research and supreme expert specialization that are of crucial significance for the development of modern pharmaceutical and chemical industry. During this 34-year period of very successful activities in the field of promoting Krka Prizes, we presented awards to 2,052 young people.
Krka Prizes are linked with International Symposiums, where invited plenary lecturers from numerous countries present their research and scientific achievements. In our International Symposiums special attention is given to the presentations of research results of Krka Prize winners. Students with Ph.D. degree participate as lecturers, other Prize winners (M.Sc. or B.A. degrees) are presenting their research results in the "Poster Session" or using Power Point presentations. The youngest Krka Prize winners, i.e. secondary school students from the Dolenjska region, will also present their awarded research works.
In the past 14 years, we have invited to Krka as plenary lecturers over 100 renowned and prominent university professors, academicians and experts (among them also a Nobel Prize Winner) from 25 countries in Europe, from the USA and Slovenia.

This year Krka has invited renowned academicians, professors and experts from six countries: Russia, Poland, Netherlands, Romania, Croatia and Slovenia.

At the 14th International Symposium, will be officially opened by the **President of the Slovenian Academy of Sciences and Arts, Academician Prof. Dr. Boštjan Žekš,**

the following plenary lecturers will take part:

Prof. Dr. Jan van der Greef from Netherlands
Prof. Dr. Jan van der Greef is scientific director of TNO's Systems Biology research within Life Sciences. In a prior responsibility as managing director of TNO Pharma, this company has become a leading organization that provides expertise, novel innovations and services to the international pharmaceutical industry throughout the drug discovery and drug development process. The emphasis on the research and contract portfolio of TNO Pharma is based on novel instrumental developments in synergy with biological/biomedical expertise. Dr. Jan van der Greef is Professor of Analytical Biosciences at Leiden University within the Leiden/Amsterdam Center for Drug Research (LACDR). His current research interest is the development of Systems Biology. He has also broadened his research on separation methods (LC, CE, CEC) combined with mass spectrometry and chemometrics into the field of analytical biosciences. He received an award for major contributions in drug analysis by the Belgium Society of Pharmaceutical sciences in 1998. He became honorary doctor at Ghent University in 2000.

Prof. Dr. Liviu Matcău from Romania
Prof. Dr. Liviu Matcău is acknowledged and world known neurologist.
He is professor chairman of Neurological Clinic at County Hospital and important university professor at University of Medicine and Pharmacy "Victor Babeş" in Timişoara.
Prof. Matcău is President of Multiple Sclerosis Foundation Timiş and member of Alzheimer's Dementia Foundation.
His scientific and research work is orientated in neuroanatomy, neurological recuperation and neurological diagnostic. He has published several books from these fields.
Professor Matcău is also member of the Romanian Academy and member of Romanian Neurological Society, Romanian Stroke Society, Timişoara Medical Association and Romanian Medical College.

Dr. Galina Vladislavovna Ramenskaya from Russia
Dr. Galina Vladislavovna Ramenskaya is Chief of the Department of clinical-pharmacological expertise of pharmaceuticals at the Ministry of Public Health, Moscow.
She received her PhD in Pharmaceutical Sciences, and is an important expert in the fields of pharmacology, clinical pharmacology and pharmaceutical chemistry.
Her current professional activities are in the fields of preclinical and clinical pharmacokinetic trials of drugs, research on bioequivalence, analysis of pharmaceuticals in drugs and biological liquids.

The scientific activity of Dr. Ramenskaya is orientated towards pharmacokinetic and pharmacogenetic trials of drug metabolism. She has published several important papers from these fields.

Prof. Dr. Maria Barcikowska from Poland

Prof. Dr. Maria Barcikowska is Head of the Department of Neurodegenerative Diseases CNS and Neurological Department, Medical Research Centre, the Polish Academy of Sciences.
Prof. Barcikowska is prominent Polish medical expert in neurology and neuropathology. Her research work is concentrated on clinical and neuropathological features of Alzheimer's disease and related disorders.
Her important research achievements are published in several important international and Polish journals.

Prof. Dr. Davorin Štimac from Croatia

Assoc. Prof. Dr Davor Štimac is Head of Department of Gastroenterology in Clinical Hospital Center Rijeka. He is also working as Assoc. Prof. for Internal Medicine at the University of Rijeka.
Prof. Štimac is an important Croatian gastroenterologist; he has accomplished his Master's Degree in pharmacology and his PhD in experimental hepatology.
He held grants and was educated in Italy and Great Britain, and having received some national and international awards for his scientific work. Dr. Štimac is a member of many international gastroenterological and hepatological associations.
He has published a variety of full papers and abstracts in international journals.

Doc. Dr. Blanka Kores Plesničar from Slovenia

Doc. Dr. Blanka Kores Plesničar is Assistant Professor of Psychiatry at the University of Ljubljana and holds the chair of Psychiatry and is Chief of the Department of Psychiatry, Teaching Hospital Maribor.
Dr. Blanka Kores-Plesničar is an important Slovenian psychiatrist. Her teaching and research activities are orientated towards psychiatry, psychotherapy, psychopharmacology and clinical pharmacology.
Dr. Kores-Plesničar is a member of Psychopharmacological Centre at the Department of Psychiatry, President of Drug Commission at the University Psychiatric Clinic, Ljubljana, member of the Clinical Investigators Team, and Slovenian representative in EMEA for efficacy, and Slovenian representative in UEMS (European Medical Society-section Psychiatry). She is also a member of important Slovenian medical associations.

Thirteen Krka Prize winners, new doctors of science, will present in a short lectures of their awarded PhD theses.
In the Poster session all other Krka Prize winners will present their research achievements obtained in their graduate and master degree studies. Research works of the youngest Krka Prize winners, secondary school students from the Dolenjska region will be also presented in this session.

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

Chairman of the Scientific Board
of 14th International Symposium
Prof. Dr. Miha Japelj

34th Krka Prizes

Programme of the 14th International Symposium

Novo mesto, 28th of October 2004

9.00 – 9.10 **Chairman's Welcome Address – Miha Japelj**
9.10 – 9.20 **Opening of the Symposium – Boštjan Žekš**

1 Plenary Lectures

Chairman: Miha Japelj

9.20 – 9.50 New Strategies in Life Sciences: Systems Biology 31
Jan van der Greef
TNO Systems Biology, LACDR, Gorlaeus Laboratories, Center for Medical Systems Biology, Leiden University, The Netherlands

9.50 – 10.20 Vascular Cognitive Impairment and the Role of Neuroprotective Agents 32
Liviu Matcău
Neurology Clinic, University of Medicine and Pharmacy, Timişuara, Romania

10.20 – 10.50 Application of Scientific Approach and Modern Knowledge in Bioequivalence Studies Of Medicines 33
Galina Vladislavovna Ramenskaya
Department of Clinical-Pharmacological Expertise of Medicines, Scientific Centre of Expertise of Medicines, Ministry of Health and Social Development of Russian Federation, Moscow, Russia

10.50 – 11.20 **Coffee Break**

11.20 – 11.50 Treatment of Alzheimer's Disease: Reality and Future 34
Maria Barcikowska
Department of Neurodegenerative Disorders, Medical Research Centre, Warsaw, Poland

11.50 – 12.20 Modern Psychopharmacology – Achievements and Promises 36
Blanka Kores Plesničar
Department of Psychiatry, University Hospital Maribor, Pivola, Slovenia

12.20 – 12.50 Psychosomatic Aspects in Gastrointestinal Diseases 37
Davor Štimac
Department for Gastroenterology, Clinical Hospital Center Rijeka, School of Medicine, University of Rijeka, Croatia

12.50 – 14.30 **Lunch Break**

2 Krka Prize Winners' Presentations

Chairman: Janko Kos

14.30 – 14.40 Incidence and Virulence of Binary Toxin Genepositive Strains of *Clostridium Difficile* and Characterization of their Toxin Locus PaLoc 41
Barbara Gerič
Biotechnical Faculty, University of Ljubljana, Slovenia
Feinberg School of Medicine, Northwestern University, Chicago, USA

17.00 – 17.10 Investigation of Nifedipine Solid Dispersion Structure Using Modulated Temperature Differential Scanning Calorimetry 55
Natalija Zajc
Faculty of Pharmacy, University of Ljubljana, Slovenia

17.10 – 17.30 **Discussion and Final Remarks**

3 Krka Prize Winners' Poster Session

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

Cleavage of Bid as a Possible Mechanism of Triggering Apoptosis by Lysosomal Cysteine Proteases 59
Tina Cirman
Faculty of Medicine, University of Ljubljana; Slovenia

Regulation of Equine Casein Gene Expression 60
Valentina Lenasi
Faculty of Medicine, University of Ljubljana, Slovenia

Neurotoxic and Enzymatic Action of *Vipera Ammodytes Ammodytes* Phospholipases A_2 61
Toni Petan
Jozef Stefan Institute, Ljubljana, Slovenia

Simultaneous Analysis of Five Genetic Risk Factors in Polish Patients with Alzheimer's Disease 62
Maria Styczynska
Polish Academy of Sciences, Warsaw, Poland

Correlative Study of Seizure Control, EEG Changes and Improvement in Cognitive Performance and Mood in Epileptic Patients Treated with Lamotrigine 63
Emilija Cvetkovska
Clinic of Neurology, Skopje, Macedonia

Prevalence and Role of *Helicobacter pylori cag*A and *ice*A Genes in Slovenian Pediatric Population 64
Matjaž Homan
University Children's Hospital, Ljubljana, Slovenia

Brand Management Under Internationalized Business Operations 65
Magda Hudoklin
Krka, d. d., Novo mesto, Slovenija

Experimental Design of Crystallization Processes 66
Miran Hvalec
Faculty of Chemistry and Chemical Technology, University of Maribor, Slovenia

Influence of Technological Parameters on Formation and Properties of Nanosuspensions 67
Petra Kocbek
Faculty of Pharmacy, University of Ljubljana, Slovenia

Information System of Quality Management in Krka 68
Mateja Košiček
Faculty of Economics, University of Ljubljana, Slovenia

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34th
Krka
Prizes

1 Plenary Lectures

New Strategies in Life Sciences: Systems Biology

J. van der Greef
TNO Systems Biology, LACDR, Gorlaeus Laboratories,
Center for Medical Systems Biology, Leiden University, The Netherlands

In Life sciences the improved ability to profile classes of biological elements (genes, proteins, metabolites) has grown tremendously and has created a shift in focus from reductionistic to holistic strategies. This has evolved into a *Systems Biology* approach, which can be defined as *studying biology as an integrated system of genetic, protein, metabolite, cellular and pathway events that are in flux and interdependent.*
Within the field of Life sciences especially within pharmaceutical and nutritional research a major emphasis of Systems Biology is on the discovery of *biomarker patterns* to provide diagnostic tools for diseases, to understand underlying mechanisms of disease, to design intervention strategies and to study health-nutrition relationships. New opportunities are created by the knowledge on the interconnection and interdependency of complex biological systems and systems thinking is gaining more and more a driver in research.
Medical/pharmaceutical oriented studies are performed on “systems” (humans : controls and patients) and perturbed systems (drug interventions) and yield system fingerprints consisting of thousands of variables (megavariate) as a function of time. In nutritional studies an even more complex situation is encountered of perturbing complex systems with complex mixtures, creating the ultimate challenge to extract information and knowledge from the fast avalanche of data created in such clinical studies. In addition the interesting aspects of the synergetic nature of components imbedded in such data structures, bridges the nutrition field via the nutraceutical field (ao herbal medicine) with the development of combinatorial pharmaceutical interventions.
The enormous challenge to tackle the data is typically approached with multivariate statistics to mine the data for explorative purposes, both in an unsupervised and supervised manner. In a next step non-linear correlations are being studied to provide information on biological connections via pathways/networks, creating system knowledge.
In this presentation the approach of Systems Biology will be discussed from the perspective of the analytical challenge on the technology/biostatistics side as well as the biology.

Vascular Cognitive Impairment and the Role of Neuroprotective Agents

L. Matcău
Neurology Clinic, University of Medicine and Pharmacy, Timişuara, Romania

Loss of memory and of cognitive function affects people worldwide. Such loss may be the result of different progressive neurological disorders of the brain. It affects both men and women and is common in the elderly. However cognitive decline is not necessarily a function of aging. Symptoms vary. For example, in severe loss of cognitive function, symptoms may include difficulty in thinking/concentrating, a drop in IQ, episodic memory loss, episodic memory gaps, amnesia, spells of aphasia, episodic problems with confusion, problems with fine/gross motor coordination, or brief dramatic changes in emotional status. Memory gaps and other cognitive problems may interfere severely with activities of daily living, such as working, or safe operation of motor vehicles. Thousands of published studies substantiate that a decline of memory/cognitive function can be controlled or even prevented. Some of these studies demonstrate that prevention can help maintain optimal brain function, while other studies show measurable benefit in reversing memory/cognitive impairment caused by normal aging or by a specific disease of aging, such as stroke. As previously stated, an inevitable consequence of aging is a reduced flow of blood to the brain. Common causes are chronic inflammation, arteriosclerosis, and increased blood "stickiness." The results of cerebral vascular disease can range from mild cognitive impairment to ischemic stroke. It is thought that symptons of age-associated memory impairment are linked with decreased cerebral circulation. Because experiments showed that ginkgo biloba may increase blood circulation to the brain, it is logical to test if ginkgo biloba extract may improve memory impairment in patients with age-associated memory impairment. Ginkgo biloba extract has demonstrated specific mechanisms of action that counteract age-related vascular disorders. Human clinical studies show that ginkgo helps to slow down or restore cognitive dysfunction in those who have vascular dementia or Alzheimer's disease. Ginkgo's therapeutic effects are thought to derive from its ability to prevent oxidative stress and its effects on peripheral and central neurotransmitter systems. Extracts of the plant contain many potentially active constituents including flavonoids, terpenoids, and phenolic acids. These have multiple and apparently synergistic pharmacologic actions, including free-radical scavenging, inhibition of membrane lipid peroxidation, vasodilation, and antihypoxia effects. Ginkgolides in the plant have antiplatelet effects. In Romania we made an open, multicentric noncomparative study regarding efficacy and safety of Ginkgo biloba (Bilobil) in patients with mild and moderate cognitive deficiency. There were enrolled 100 patients with age over 50 years from 7 universitary centers, they received 120 mg/day of Bilobil. Tinnitus, dizziness and simptoms of cognitive impairment (the Cognitive Subscale of the Alzheimer's Disease Assessment Scale, or ADAS-Cog) were monitorized at the beginning of the study, after one, two and three months of treatment. Bilobil improves cognitive performance regarding memory and attention, raises spell coherence, clarity in thinking, significant reduce tinnitus and dizziness. Side effects are rare with the use of ginkgo extract. In less than 1% of patient there appeared mild gastrointestinal symptoms. It has been reported that in people with cerebral insufficiency (poor blood flow to the brain) a mild headache or some dizziness may follow the first couple of days of using of ginkgo extract. Bilobil is a safe medication, easy to administrate and the patients's compliance is very good.

Application of Scientific Approach and Modern Knowledge in Bioequivalence Studies of Medicines

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Scientific Centre of Expertise of Medicines,
Ministry of Health and Social Development of Russian Federation, Moscow, Russia

Estimation of drugs' bioequivalence (BQ) is the basic type of medical-biological control of generic drugs. BQ studies allow to make justified conclusions concerning quality of compared drugs relative to efficiency and safety using smaller volume of initial information and in shorter periods of time, compared to clinical studies.

In recent years amount of generic drugs has sharply increased leading to increasing of BQ studies. At present, the rules of conducting such studies which are close to European requirements have been stated in Russia.

A BQ study can be subdivided into two stages: clinical and pharmacokinetic (laboratory). Quality of conducting of each of these stages influences the validity of results.

One of the essential factors is the number of volunteers in a study. It is well known, that each drug has a chracteristic intra- and inter-individual differences of concentration values (variation coefficient). For some drugs it may be quite small, for others – rather large and for some it may be very high. As it has been accepted in our country, the minimal number of volunteers sufficient to provide a statistically valid study amounts to 18 people. More volunteers may be required to compare drugs having significant variability of pharmakokinetic parameters. In planning an experiment necessary values for calculation of the number of volunteers can be derived (estimated) from analogous studies, scientific literature data, "pilot" studies results. Nevertheless, there are cases when after conducting a study a question of increasing the number of volunteers arises, or the number of volunteers is very high initially. Taking into account that differences in values of pharmacokinetic parameters depend first of all on features of drugs' metabolism and individual condition of a biotransformation system, in our scientific work devoted to pharmacokinetic and pharmacogenetic study of drugs' metabolism, we pay special attention to possible methods of estimation of metabolic enzymes activity in an individual.

In our work it was shown that inclusion of volunteers based on CYP2D6 polymorphism definition results allows to decrease by three times differences in concentrations' values of drugs, metabolized by the isozyme. In the study volunteers with equal genetically determined activity of the isozyme are included. Besides marker preparations are successively used in estimation of activity of this or that isozyme.

One more factor which is influencing BQ result attracts much attention. That is choice of comparative drug. It is known that the majority of sources recommend to choose the original drug for this purpose. But this is not always possible (the drug may not be registered, not supplied etc.) That is why scientific studies including collecting data for justified use of generic drugs (those which have proven to be good in clinical practice and whose equivalence to the original drug has been proved in studies and in practice) as comparative ones are being conducted.

Drugs produced by Krka, Slovenia, can be the main example. Medicine preparations of this firm have demonstrated good results in clinical practice Russia, that is why they are often chosen as standards by Pharmacological Committee. For example, such preparations as Cordipin, Enap, Pentilin, Erason, Pyrasinamide, Lanzoptol and others are permanently used as comparative preparations.

In general, it can be noted, that application of scientific achievements in the field of genetics, chemistry, biology and modern methods of investigations permit us to increase and optimize quality of clinical studies as well as BQ studies.

Treatment of Alzheimer's Disease: Reality and Future

M. Barcikowska
Department of Neurodegenerative Disorders, Medical Research Centre, Warsaw, Poland

Alzheimer's disease (AD) is a progressive neurodegenerative disorder that affects a large proportion of the elderly population, causes a progressive decline in memory and other cognitive functions. The neuropathologic hallmarks of AD are neurotic plaques and neurofibrillary tangles (NFT). Other hallmarks are inflammation, neuronal and synaptic loss and changes in the neurotransmitter system.
There is no effective treatment in AD even though a great effort was done in that field. New therapeutic approaches-including those more closely targeting at the pathogenesis of the disease will be discussed. According to Evidence Based Dementia Practice only inhibitors of acetylocholinesterase (AChE) are approved in mild and moderate stages of AD treatment. From 1970 it is widely believed that AD is the disturbance of the acetylocholine function in the brain. From the end of the year 2003, FDA also approved memantine for more severe phases of AD (Reisberg et al. 2000)

Treatment based on the amyloid cascade hypothesis:

Although the relationship between senile plaques and NFT is still controversial, in the past few years several studies have been published supporting the pivotal role played by amyloid in neurodegeneration, and research into AD has been guided by the so-called "amyloid cascade hypothesis". The deposition of amyloid senile plaques seems to primarily defect driving AD pathogenesis and NFTs as well as other pathological changes.

APP metabolism as a target…

Even though there are several strategies aimed at acting directly on Aβ as a causative agent for AD, no causative medicine is still available. Numerous laboratories are currently investigating β- and γ-secretase, the two amyloidogenic proteases that cleave the Aβ peptide out of the amyloid precursor protein (APP). Secretase inhibitors are being studied for AD on the strength of the amyloid hypothesis, which posit that abnormal accumulation of Aβ and a cascade of neurotoxic effects and eventually lead to neurodegeneration and the development of AD. By altering the processing of amyloid precursor protein (APP), β- and γ- sercretase enzymes are believed to enhance the production of amyloid in the brain.

Aβ oligomers

Amyloid beta peptides implicated in the pathogenesis of AD particularly as oligomers that are correlated with Aβ cellular toxicity. The initial phase of oligomerization involves formation of pentamer/ hexamer units, paranuclei, that then associate to form large oligomers and protofibrils. Paranuclei formed by Aβ 42 may be important therapeutic targets per se. Inhibition of oligomers as toxic forms of Aβ has therefore emerged as one approach to the treatment of AD.

Immunotherapy and Alzheimer's disease

The Elan vaccine, referred to as AN-1792 is a synthetic version of the Aβ (Schenk et al 1999). The vaccine has shown some promising results in slowing the progression of AD, as the results of a small, preliminary follow-up study suggest. Vaccine tests have been promising in transgenic mice, but the first trial of the vaccine in humans (300 patients all over the world) was stopped before time because of severe side effects as an aseptic meningoencephalitis, which in two cases caused death of treated patients. Because of these serious side effects, administration of the vaccine, developed by the Elan Corporation was halted in all centers.

Aβ breakers

Soto et al. (2001) have engineered short synthetic peptides homologous of the central hydrophobic region of Aβ that should be active in disrupting the β- sheet stabilization. The peptide has two unique features, it crosses BBB and does not induce antibody production.

Metal chelators

Bush et.al. (2003) have pointed out the critical role played by heavy metals in AD suggesting that endogenous metal ions such as zinc, copper or iron may contribute to the aggregation of Aβ. Based on this theory an innovative therapy using a drug called clioquinol (a hydrophobic Cu/Zn chelator) showed some early promise in slowing the progressive memory loss of Alzheimer's.

Modulation of tau (and APP) metabolism

NFTs are a distinguishing neuropathological feature found in postmortem brain with AD. The density of these lesions correlates with the severity of AD. Tau phosphorylation is believed to initiate or facilitate dissociation from microtubules destabilization, the decay of cellular transport properties, and cell death. Key challenges in developing effective therapeutic agents include identification of the relevant kinases responsible for aberrant tau phosphorylation in AD, synthesis of inhibitors selectively targeting those kinases.

Aβ peptides are derived from APP sequential proteolysis catalyzed by the aspartyl protease BACE, followed by presenilin- dependent γ-secretase cleavage. Presenilin interacts with nicastrin APH 1 and pen2, α-catenin β-catenin nad glycogen synthase kinase 3β (GSK3β), but the functional role of these proteins in γ-secretase activity has not been established.

Modern Psychopharmacology – Achievements and Promises

B. Kores Plesničar
Department of Psychiatry, University Hospital Maribor, Pivola, Slovenia

The last decade has truly been a remarkable one for biomedical research. The »molecular medicine revolution« has brought to bear the power of sophisticated cellular and molecular biological methodologies to tackle many of society's most devastating illnesses. In this way, psychiatry has entered a new and exciting era demarcated by current rapid advances and future promises of genetics, molecular and cellular biology and improving technologies. At the moment there is a progress in the understanding of psychiatric illnesses. There are disorders of synapses and circuits rather than purely abnormalities in individual neurotransmitters. Unfortunately, clinical translation of these findings towards a direct benefit to patients who suffer from severe psychiatric diseases has not been so fast. But many scientists believe that the next significant advances in psychiatric treatment will occur not within the context of further neurotransmitter manipulation, but perhaps in other areas, such as promoting neurogenesis. Important findings in genetics, epigenetics, proteomics, neuroimaging, new specific endophenotypes and animal models represent changes in the basic neuroscience that will probably have an important impact on our understanding of the biological background of psychiatric diseases and on the development of novel or improved therapies.

But we are still captured in the present time. Exactly 50 years ago Lehman published the first study of chlorpromazine. With regard to the case of chlorpromazine, we should never forget that the real breakthroughs in psychopharmacology were generally achieved on the basis of careful clinical observations only in a very small number of psychotic patients. A number of mechanisms of action of psychopharmacological agents have been explored during the last 30 years and we are still walking in a circle. Although there have been improvements in the diagnosis and management of patients with some of the important psychiatric disorders in recent years, many challenges remain. Currently available pharmacotherapies do not fully meet the needs neither of patients nor psychiatrists. Barriers to achieving satisfactory clinical outcomes for both the acute and long-term treatments of many psychiatric illnesses still exist. The challenge for a successful and efficacious use of psychopharmacological agents is in finding the individual drug that would fit the needs of an individual patient best. This must be our primary goal.

Psychosomatic Aspects in Gastrointestinal Diseases

D. Štimac
Department for Gastroenterology, Clinical Hospital Center Rijeka,
School of Medicine, University of Rijeka, Croatia

Functional gastrointestinal disorders result mostly from dysregulation of «brain-gut» neuroenteric systems. The «brain-gut» axis consists of bidirectional neural pathways that link cognitive and emotional centers in the brain with the neuroendocrine centers, immune system and the enteric nervous system. Regulation of these connections occurs by various not site specific neurotransmitters.
Patients with functional GI disorders exhibit increased motor reactivity to several stressors and have decreased pain thresholds. In a predisposed individual, stress can result increased responsiveness of central stress circuits and vulnerability to the development of functional and affective disorders.
Some gastrointestinal disorders like irritable bowel syndrome, esophageal motility disorders and non-ulcer dyspepsia are commonly associated with psychiatric comorbidity that may or may not be etiologic.I n other diseases like inflammatory bowel disease (IBD) or chronic viral hepatitis there is consideration of the emotional consequences of gastrointestinal diseases. Eating disorders like anorexia nervosa, bulimia nervosa and rumination disorder do not appear to be either entirely physiologic or psychological in origin but instead probably represent an interplay between two.
In patients with functional, but also in those with organic diseases psychosomatic aspects are very important because psychosocial factors may influence symptoms and behaviour in ways that are not understood in terms of disease or treatable by disease-specific measures.

34. Krkine nagrade
34th Krka Prizes

2 Krka Prize Winners´ Presentations

Pogostost, virulenca in opis toksinskega lokusa PaLoc sevov bakterije *Clostridium difficile* z zapisom za binarni toksin

B. Gerič
Biotehniška fakulteta, Univerza v Ljubljani, Slovenija
Feinberg School of Medicine, Northwestern University, Chicago, ZDA
Mentor: M. Grabnar
Somentorica: M. Rupnik
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Bakterija *Clostridium difficile* je povzročitelj črevesnih infekcij po terapiji z antibiotiki. Sevi *C. difficile* lahko delajo toksine TcdA, TcdB in/ali CDT. TcdA in TcdB veljata za glavna dejavnika virulence. Toksin CDT dela manjše število sevov in je manj raziskan. Spada v skupino klostridijskih binarnih toksinov, v celici deluje na aktin kot ADP-ribozil transferza in poruši citoskelet. Citotoksičen je za celice v kulturi, njegova vloga pri razvoju bolezni ni jasna. V tem delu smo določili (i) pogostost sevov, ki delajo CDT, (ii) testirali virulenco sevov, ki delajo izključno CDT ter (iii) določili zgradbo toksinskih lokusov. Prisotnost genov za toksin CDT smo pokazali pri 2-6% sevov. Virulenco smo testirali na dveh živalskih modelih. Akumulacija tekočine na modelu prevezane zanke črevesa zajca nakazuje vlogo CDT pri razvoju bolezni. Pri skupini sevov, ki izdeluje samo CDT, smo določili zgradbo toksinskega lokusa PaLoc, ki je v primerjavi z referenčnim sevom močno spremenjen. Pri različnih sevih smo pokazali obsežno delecijo v lokusu CDT, ki onemogoči sintezo aktivnega proteina, binarnega toksina CDT. Naši rezultati nakazujejo, da je CDT dodatni virulenčni dejavnik sevov *C. difficile*. Študije CDT pozitivnih izolatov so pomembne pri načrtovanju diagnostičnih strategij, ki zaznajo toksinske gene *C. difficile* in njihove produkte. S trenutnimi diagnostičnimi testi TcdA-TcdB-CDT+ seve v diagnostičnih laboratorijih označijo kot nenevarne.

Incidence and Virulence of Binary Toxin Genepositive Strains of *Clostridium Difficile* and Characterization of their Toxin Locus PaLoc

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Clostridium difficile is a causative agent of intestinal infections after antibiotic treatment. Strains of *C. difficile* can produce three different toxins: TcdA, TcdB and/or CDT. TcdA and TcdB are believed to be the main virulece factors. Toxin CDT, a clostridial binary toxin, is produced by a smaller number of strains. CDT acts as actin-specific ADP-ribosyltransferase. It is cytotoxic for cultured eukaryotic cells but its role in the development of disease is not well understood. In this work we (i) determined the frequency of strains that produce CDT, (ii) tested the virulence of strains that produce only CDT, and (iii) determined the structure of toxin locus. We showed the presence of CDT genes in 2-6% of strains. Virulece was tested in two different animal models. Fluid accumulation in rabbit ileal loop suggests a role of CDT in pathogenesis. In strains, which produce only CDT, we have determined the structure of PaLoc with extensive changes compared to the reference strain. In different strains, we have showed an extensive deletion which disables the synthesis of active protein, binary toxin CDT. Our results suggest that CDT is an additional virulence factor of *C. difficile*. Characterization of binary toxin-positive isolates is important considering potential diagnostic strategies targeting toxin genes and their products. With the current diagnostic tests TcdA-TcdB-CDT+ strains are reported as nontoxigenic.

Stereoselektivne sinteze in pretvorbe v vrsti terpenskih enaminonov

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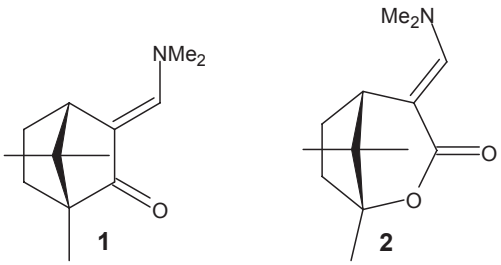
Pri našem delu smo s propenoatno metodologijo iz (+)-kafre pripravili dva terpenska enaminona, (1*R*,3*E*,4*S*)-3-[(dimetilamino)metiliden]-1,7,7-trimetil[2.2.1]heptan-2-on (**1**) in (1*R*,4*E*,5*R*)-3-[(dimetil-amino) metiliden]-1,8,8-trimetil-2-oksabiciklo[3.2.1]oktan-3-on (**2**), ter proučevali reakcije le-teh z nukleofili in elektrofili ter nadaljnje pretvorbe nastalih produktov. Tako smo z nukleofili pripravili produkte izmenjav, ki smo jih naknadno ciklizirali, oksidirali, reducirali, tvorili komplekse in jih uporabili kot dipolarofile ali 1,3-dipole pri cikloadicijah. Z elektrofili smo pripravili (1*R*,4*E*,5*S*)-1,8,8-trimetil-2-oksabiciklo[3.2.1]oktan-3,4-dion 4-oksim in (1*R*,4*E*,5*S*)-1,8,8-trimetil-4-{[metil(nitrozo)-amino]metiliden}-2-oksabiciklo[3.2.1]oktan-3-on. Nadalje smo proučevali reakcije oksima z Grignardovimi reagenti in redukcije.



1 **2**

Stereoselective Synthesis and Transformations in the Series of Terpene Enaminones

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In our research work two terpene enaminones, (1*R*,3*E*,4*S*)-3-[(dimethylamino)methylidene]-1,7,7-trimethyl[2.2.1]heptan-2-one (**1**) and (1*R*,4*E*,5*R*)-3-[(dimethylamino)methylidene]-1,8,8-trimethyl-2-oxabicyclo[3.2.1]octan-3-one (**2**), were prepared from (+)-camphor using propenoate methodology. Reactions of terpene enaminones with various nucleophiles and electrophiles, and their further transformations were studied. Thus, with nucleophiles exchange products were formed, which were subsequently cyclized, oxidized, reduced, used as ligands in complexes and as dipolarophiles or 1,3-dipoles in cycloadditions. Reactions with electrophiles gave (1*R*,4*E*,5*S*)-1,8,8-trimethyl-2-oxabicyclo[3.2.1]octan-3,4-dion 4-oxime and (1*R*,4*E*,5*S*)-1,8,8-trimethyl-4-{[methyl(nitroso)amino]-methylidene}-2-oxabicyclo[3.2.1]octan-3-one, respectively. Further reactions of oxime with Grignard reagents and reductions were studied.

Vpliv Cr(III) na znotrajcelične procese v prokariontskih in evkariontskih celicah

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Cr(III) je mineralni element, bistven za nemoten metabolizem ogljikovih hidratov in lipidov v telesu, v nekaterih oblikah pa lahko deluje škodljivo. V predstavljenem delu smo z različnimi metodami preučevali vlogo Cr(III) v prokariontskih in evkariontskih celicah ter preverjali njegov vpliv na DNA in na delovanje nekaterih esencialnih encimov in vitro. Rezultati so pokazali, da Cr(III), v odvisnosti od ligandov, ki so nanj vezani, lahko vstopa v bakterijske celice, kjer povzroči topološke spremembe v bakterijskem kromosomu ter poveča tvorbo ROS in tako vodi v nastanek oksidativnih poškodb DNA. Podobno kot v bakterijskih celicah Cr(III) deluje tudi v kvasovki S. cerevisiae S288c, kjer inducira prepis genov RNR3, HSP104, GSH1 in GTT1. V obliki Cr(III)-pikolinata prepreči podvajanje DNA v humanih celicah, v višjih koncentracijah pa aktivira proces kontrolirane celične smrti. In vitro se Cr(III) veže na bakterijsko girazo in humano topoizomerazo IIα ter tako ovira njuno aktivnost sproščanja dodatno zvite plazmidne DNA, v kombinaciji z askorbatom in H_2O_2 pa povzroča enoverižne in dvoverižne lome plazmidne DNA. Poškodbe so posledica $^{\cdot}OH$, nastalih med Cr(II) ↔ Cr(III) redoks cikliziranjem, njihova intenziteta pa je odvisna od zvrsti Cr(III), razmerja med Cr(III) ter reducentom in od sestave medija, v katerem reakcija poteka. Rezultati predstavljene študije kažejo na kompleksno vlogo Cr(III) v bioloških sistemih in opozarjajo na problematiko Cr(III)-vsebujočih mineralnih dodatkov, predvsem z vidika njihove varnosti. Vezava Cr(III) na celične makromolekule spremeni izražanje genov in delovanje nekaterih encimov, hkrati pa kompleksacija različnih ligandov na Cr(III)-center lahko spremeni redoks potencial Cr in tako omogoči aktivno sodelovanje tega elementa v celičnih redoks reakcijah. Nastale ROS vodijo v oksidativne poškodbe DNA, lipidov in proteinov v celicah ter posledično v razvoj različnih bolezni in prezgodnje staranje.

The Influence of Cr(III) On Intracellular Processes in Prokaryotic and Eukaryotic Cells

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Trivalent chromium is an essential element needed for proper carbohydrate and lipid metabolism. In some forms and in high concentrations, however, it can be harmful. In the presented research the effects of Cr(III) on prokaryotic and eukaryotic cells were investigated and also the influence of Cr(III) on isolated DNA and some selected essential enzymes were studied. The results indicate that, depending on the ligands bound to Cr-center, Cr(III) can enter bacterial cells. Once inside, Cr(III) binds to the bacterial chromosome, causing topological changes and also ROS formation with the consecutive oxidative DNA damage. The influence of Cr(III) is similar also in the yeast S. cerevisiae S288c as shown by the real time RT-PCR measurements of RNR3, HSP104, GSH1 and GTT1 gene transcription. In the form of Cr-picolinate, Cr(III) has antiproliferative effects and induces apoptosis in human U937 cells, indicating severe DNA damage and/or irreversible cell cycle block. In vitro Cr(III) hinders the relaxation activities of both bacterial gyrase and human topoisomerase IIα. The inhibition is partially the consequence of Cr(III) binding to the DNA, but is also caused by the direct binding of Cr to the enzymes. In in vitro test system with ascorbate and H_2O_2, Cr(III) in the form of Cr-picolinate produces single strand and double strand DNA breaks which are caused by hydroxyl radicals formed during Cr(II) ↔ Cr(III) redox cycling in the presence of ascorbate and H_2O_2. The intensity of DNA lesions is dependent on the ratio between Cr-picolinate and reductant and also on the reaction media composition. The presented results outline the complex role of Cr(III) in the biological systems and drawl the attention to the Cr(III)-nutritional supplements, especially with regards to their safety. Inside the cells, the binding of Cr(III) to the

biological macromolecules causes changes in gene expression and enzyme activities. The complexation of different organic ligands to Cr(III) can enable the active participation of Cr in the cell redox reactions leading to ROS–induced oxidative damage of DNA, proteins and lipids, and consequently to different diseases and premature aging.

Diels–Alderjeve reakcije nekaterih 2*H*-piran-2-onov in pripojenih piran-2-onov

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Disertacija predstavlja serijo Diels–Alderjevih reakcij med substituiranimi 3-benzoilamino-2*H*-piran-2-oni in njihovimi pripojenimi analogi ter različnimi dienofili: alkeni (npr. maleinanhidrid,[1] *N*-substituirani maleimidi[2] in 2-metoksifuran) in z alkini (fenil-, difenilacetilen, etil propinoat, dimetil- in dietil acetilendikarboksilat).[3] Glede na substituente vezane na izhodne 2*H*-piran-2-one smo izolirali različne produkte; reakcija z alkeni kot dienofili stereospecifično daje biciklo[2.2.2]oktenske sisteme; nekateri izmed njih vsebujejo še ne opisane vzorce substituentov. Pri bicikličnih, pripojenih piran-2-onih smo opazili močan vpliv 5-okso skupine – reakcijo namreč usmerja v nastanek benz[*e*]izoindolov. To je nova, nenavadna aromatizacija, gnana z učinkom substituentov. To aromatizacijo lahko izvedemo tudi z dodatkom Rh/C, kar predstavlja še neopisano uporabo tega katalizatorja. Celo 5-nesubstituirani pripojeni piran-2-oni in monociklični 2*H*-piran-2-oni, s katerimi sicer nastanejo biciklo[2.2.2]oktenski produkti, pod vplivom Rh/C tvorijo benz[*e*]izoindole. Reakcije z alkini, pri katerih nastanejo multifunkcionalno substituirani anilini, so predstavljene kot primer študije vplivov substituentov na reaktivnost. Reakcije z alkini smo raziskali tudi pod visokotlačnimi pogoji. Predstavljena je tudi nenavadna reakcija z 2-metoksifuranom pri kateri stereo- in regiospecifično nastane oksabiciklo[2.2.2]oktenski adukt, ki vsebuje CO_2 mostiček.

Diels–Alder Reactions of some 2*H*-Pyran-2-ones and Fused Pyran-2-ones

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The thesis presents a set of Diels–Alder reactions between highly substituted 3-benzoylamino-2*H*-pyran-2-ones and their fused analogues reacting with different dienophiles: alkenes (for example maleic anhydride,[1] *N*-substituted maleimides[2] and 2-methoxyfuran) and alkynes (phenyl-, diphenylacetylene, ethyl propinoate, dimethyl- and diethyl acetylenedicarboxylate).[3] According to the substituents on the starting 2*H*-pyran-2-ones different products were isolated: alkenes as dienes stereospecifically yielded bicyclo[2.2.2]octene systems, some of them containing novel substitution patterns. With bicyclic fused pyran-2-ones we found a strong effect of the 5-oxo group – it directs the reaction in a novel unusual substituent-driven aromatization toward benz[*e*]isoindoles. On the other hand, this aromatization can also be achieved in an unprecedented catalysis with Rh/C. And even 5-unsubstituted fused pyran-2-ones, as well as monocyclic 2*H*-pyran-2-ones, which without the dehydrogenation catalyst produce bicyclo[2.2.2]octenes, can be directed toward the synthesis of benz[*e*]isoindoles when Rh/C is added. Reactions with alkynes produce multifunctionally substituted anilines. The emphasis was on the application of high-pressure techniques and evaluation of substituents effects on the reactivity. An unusual reaction with 2-methoxyfuran is also presented, stereo- and regiospecifically producing oxabicyclo[2.2.2]octene derivatives which contain CO_2 bridge.

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Vpliv kalcijevega antagonista amlodipina na aktivnost sintaze dušikovega oksida v endoteliju arterij prašiča

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Kalcijevi antagonisti iz skupine dihidropiridinov (DHP) naj bi povečali sproščanje dušikovega oksida (NO) iz endotelija. Endotelijske celice na svojih membranah nimajo napetostno odvisnih Ca^{2+}-kanalov, zato ostaja mehanizem delovanja DHP na endotelij nepojasnjen. V naši raziskavi smo želeli preveriti domnevo, da amlodipin vpliva na od endotelija odvisno relaksacijo, ki jo posreduje NO, ter osvetliti možne mehanizme njegovega delovanja na encim endotelijsko sintazo NO (eNOS).
Na obročkih sveže izolirane kontrahirane koronarne arterije prašiča z ohranjenim endotelijem je amlodipin izzval od endotelija odvisno relaksacijo, ki jo posreduje NO, ter premik krivulje odnosa med koncentracijo in relaksacijo, izzvano z bradikininom (Bk), v levo. S pomočjo elektronske spinske resonančne spektroskopije smo pokazali, da amlodipin poveča koncentracijo sproščenega NO iz sveže izolirane koronarke, kar je lahko posledica povečane aktivnosti eNOS. Z metodo "Western blot" smo pokazali, da je bil v kulturi nestimuliranih endotelijskih celic serinski ostanek v eNOS (Ser^{1177}) nefosforiliran, treoninski (Thr^{495}) pa fosforiliran (encim je v tem stanju neaktiven), pod vplivom amlodipina pa se je povečala fosforilacija Ser^{1177} in zmanjšala fosforilacija Thr^{495}, kar je skladno s povečano aktivnostjo encima. Najverjetneje gre za vpliv na proteinsko kinazo C (PKC), saj je dodatek amlodipina v kulturo endotelijskih celic značilno zmanjšal fosforilacijo PKC, kar je skladno z zmanjšano aktivnostjo PKC. Znano je namreč, da aktivna oblika PKC inhibira aktivnost eNOS.
Rezultati naše raziskave nakazujejo, da amlodipin poveča aktivnost eNOS tako, da povzroči spremembe fosforilacije Ser^{1177} in Thr^{495} v eNOS, ki so najverjetneje posledica inhibicije PKC. Tako nastaja več NO, zato je od endotelija odvisna vazodilatacija pod vplivom delovanja amlodipina večja.

Effect of a Calcium Antagonist Amlodipine on the Activity of Nitric Oxide Synthase in Endothelium of Porcine Arteries

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Dihydropyridine (DHP) calcium antagonists are reported to increase the release of nitric oxide (NO) from native and cultured endothelial cells. Since endothelial cells do not express voltage-dependent Ca^{2+} channels, the cellular mechanisms underlying this phenomenon are unclear. Thus, the aim of our study was to investigate whether amlodipine, a Ca^{2+}channel blocker is able to induce an endothelium-dependent, NO-mediated relaxation and to determine whether it can influence the activity of the endothelial NO synthase (eNOS) by altering its phosphorylation.
In isolated, precontracted, endothelium-intact porcine coronary arteries, amlodipine elicited a NO-mediated relaxation and a leftward shift in the concentration-relaxation curve to bradykinin (Bk). Furthermore, amlodipine increased the generation of NO from freshly isolated arterial segments, as detected by electron spin resonance spectroscopy. Using "Western blot" method, we showed that in the culture of unstimulated endothelial cells, eNOS was not phosphorylated on Ser^{1177} but was phosphorylated on Thr^{495} (the unactive form of the enzyme). Amlodipine elicited the phosphorylation of Ser^{1177} and attenuated Thr^{495} phosphorylation, with a time course similar to that of eNOS activation. Amlodipine time-dependently attenuated the phosphorylation of protein kinase C (PKC) and hence its activity, with a time course similar to the changes in eNOS phosphorylation. Namely, it is known that the active form of PKC inhibits the activity of eNOS.
In conclusion, the results of our study indicate that the Ca^{2+}-antagonist, amlodipine, enhances endothelial NO generation by inducing changes in the phosphorylation of eNOS on Ser^{1177} and Thr^{495}. The effect of amlodipine on eNOS is probably mediated by the inhibition of PKC. Along with enhanced production of NO goes an endothelium-dependent vasodilation under the influence of amlodipine.

Lithiation of Phenylpropylene Oxides: Synthetic Usefulness[1]

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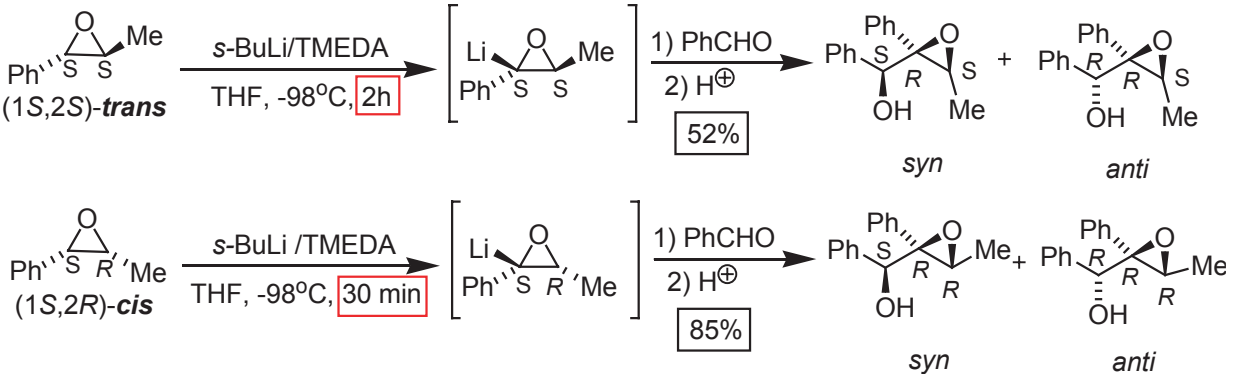
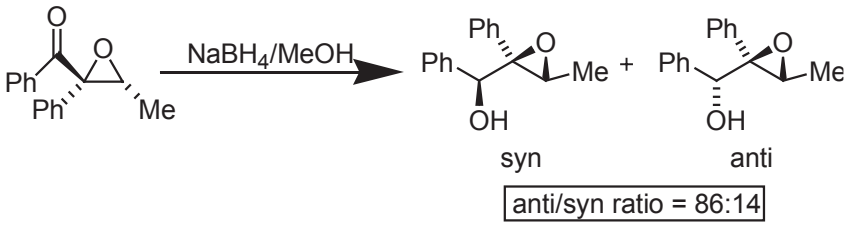
Optically active phenylpropylene oxides are highly versatile building blocks for asymmetric syntheses[2] and have been, recently, also successfully employed as key intermediates in the stereoselective construction of multisubstituted tetrahydrofurans[3] whose unit occurs frequently in natural products, e.g., antibiotics and C-glycosides. In the course of my experimental thesis in Organic Chemistry I investigated a new route to optically active more functionalized phenylpropylene oxides exploiting the nucleophilic character of optically active α-lithiated styrene oxides, such as (1S, 2S)-(-)- and (1S, 2R)-(+)-phenylpropylene oxides.

(1*S*,2*S*)-(-)-*trans*phenylpropyleneoxide (1*S*, 2*R*)-(+)-*cis*phenylpropyleneoxide

The diastereomeric lithiated epoxides proved to be, in donor solvents and in the presence of ligands such as TMEDA, both chemically and configurationally stable so that they could be successfully and stereospecifically captured with electrophiles (e.g. D_2O, MeI, allyl bromide, acetone, N,N-dimethylbenzamide) to give more substituted stereodefined styrene oxide derivatives. Interestingly, *cis*-phenylpropylene oxide resulted to be more reactive with respect to the *trans* isomer; indeed, the former required only 30 min for its α-lithiation whereas the latter at least 2h. Both these lithiated epoxides also reacted smoothly with PhCHO leading in good yields to a mixture of the corresponding syn/anti adducts although with poor diastereoselectivity at the newly created stereogenic center. Neverthless, the two diastereomeric epoxy alcohols could be fully separated by preparative HPLC and spectroscopically characterized with reference to their relative and absolute configuration.



Since the stereoselective synthesis of α,β-epoxy alcohols is a challenging problem in synthetic organic chemistry, as they are excellent starting materials for the preparation of stereodefined polyols and biologically active compounds, we decided to study the carbonyl reduction of the α,β-epoxy ketone derived from the coupling reaction of *cis*-Li with *N,N*-dimethylbenzamide. Results showed that no stereoselective improvements were achieved when the reduction with $NaBH_4$/MeOH was carried out in the presence of Lewis acids such as $CaCl_2$, $ZnCl_2$ and $CeCl_3$, thus excluding the possibility of a chelated cyclic transition state. The observed *anti* diastereoselectivity was, instead, accounted for with a modified open Felkin-Ahn model. More mork is currently underway on the reduction of the α-benzoyl moiety in the case of the *trans*-substituted epoxide.



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Vsebnost saponinov v tkivni kulturi pomladanskega jegliča (*Primula veris* L.)

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Korenine pomladanskega jegliča (*Primula veris* L.) vsebujejo znatne količine triterpenskih saponinov, ki delujejo kot ekspektoransi in se dodajajo v sirupe za izkašljevanje. Vzpostavili smo različne tipe tkivnih kultur pomladanskega jegliča: uspešen sistem za mikropropagacijo, kulturo kalusa, celično suspenzijsko kulturo in inducirali somatsko embriogenezo. Pomladanski jeglič smo poskušali transformirati z bakterijo *Agrobacterium rhizogenes*, da bi pridobili hitro rastočo laskasto koreninsko kulturo. Gene za indukcijo korenin, *rol* A, B, C, smo poskušali vnesti tudi z bakterijo *Agrobacterium tumefaciens* in biolistiko. Laskastih korenin nismo uspeli inducirati, dokazali pa smo prehodno izražanje gena za β-glukuronidazo po transformaciji z bakterijo *Agrobacterium tumefaciens* LBA 4404 v vseh uporabljenih izsečkih (list, listni pecelj, vršiček, korenina) in po streljanju plazmida pBI 221 s pomočjo genske puške v izsečkih korenin. V vseh dobrorastočih tkivnih kulturah smo s tekočinsko kromatografijo visoke ločljivosti z obrnjeno fazo (RP-HPLC) kvantitativno ovrednotili vsebnosti primula kisline I (PK I). V vseh vzpostavljenih kulturah smo določili visoko vsebnost PK I, ki je dosegla v kulturah z največjo vsebnostjo naslednje vrednosti: v koreninah rastlin, pridobljenih z *in vitro* mikropropagacijo 2,74% suhe mase, v kalusu 1,19% suhe mase, v celični masi celične suspnzijske kulture 1,18% suhe mase in v strukturah, podobnim somatskim embrijem 5,89% suhe mase. V kulturi, somatskim embrijem podobnih struktur z najvišjo vsebnostjo PK I je bila ta 40% višja, kot je povprečna vsebnost v koreninah v *in vivo* razmerah rastočih rastlin, ki se konvencionalno uporabljajo za pridobivanje primula saponinov.

The Content of Saponins in Tissue Culture of *Primula veris* L.

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Roots of *Primula veris* L. contain considerable amounts of triterpene saponins that act as expectorants. In the pharmaceutical industry, they are added into syrups for treating bronchial ailments. Various types of tissue culture of *Primula veris* L. have been established: an efficient system for micropropagation, callus culture, suspension culture and induction of somatic embryogenesis. The bacterium *Agrobacterium rhizogenes* was used to try to transform *P. veris* and to obtain fast growing hairy root cultures. Attempts were also made to introduce genes for root induction, *rol* A, B and C, into the plant cell by bacterium *Agrobacterium tumefaciens* and by particle bombardment. Hairy root culture could not be established. However, transient expression of the gene for β-glucuronidase was detected after transformation by bacterium *Agrobacterium tumefaciens* LBA 4404 in all the explants used (leaf, petiole, apex, root) and, after particle bombardment of plasmid pBI 221, in some root explants. In strongly growing tissue cultures, the content of primula acid (PK I) and the presence of other primula saponins were determined by reversed phase-high performance liquid chromatography (RP-HPLC). The content of PK I was high in all established cultures and the highest contents in different tissue cultures were: in roots of plantlets obtained from the micropropagation system 2,74% of dry mass, in callus 1,19% of dry mass, in biomass of cell suspension culture 1,18% of dry mass and in structures like somatic embryos 5,89% of dry mass. The highest content of PK I in structures like somatic embryos was 40% higher than the average content of PK I in roots of *in vivo* grown plants, which are used conventionally for extraction of primula saponins.

Biopharmaceutical Characterization of Paracetamol Extended Release Matrix Tablets Based on Novel Carbopol® Polymers

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Biopharmaceutical characterization of the drug product includes the *in vitro* and *in vivo* evaluation of drug product in order to identify the mechanism of drug release from the dosage form; identify the factors that could affect drug release kinetics both *in vitro* and *in vivo*; determine drug release profile and resulting bioavailability *in vivo*; define the *in vitro* experimental conditions that would be predictive of drug products *in vivo* behaviour and establish quantitative *in vitro – in vivo* correlation (IVIVC). Present investigation was focused on the evaluation of novel polyacrilate polymers, Carbopol 971P and Carbopol 71G as the sustained release agents in hydrophilic matrix tablet formulation. Following the results of the *in vivo* study in a group of volunteers, as well as the extensive *in vitro* testing using various experimental conditions, meaningful level A IVIVC was established. Different IVIVC methodologies were evaluated, based on deconvolution and/or convolution approaches, using linear regression analysis as well as the nonlinear proportional odds, proportional hazards and proportional reversed hazards models and Artificial Neural Networks. Artificial Neural Network analysis resulted with distinctly high level A IVIVC, indicating the superiority of the proposed methodology, its ability to generalize complex relations among the input and output parameters and its applicability in the evaluation of *in vitro* and *in vivo* results in order to predict drug products *in vivo* behaviour based on the *in vitro* drug release data.

Stereoselektivne in regioselektivne sinteze in pretvorbe nekaterih γ-laktamov in γ-laktonov

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V prvem delu raziskovalnega dela smo študirali 1,3-dipolarne cikloadicije diazometana in nitril oksidov na metil (*S*)-3-[(*E*)-cianometiliden]-2-oksotetrahidrofuran-5-karboksilat in metil (*S*)-1-*terc*-butoksikarbonil-3-[(*E*)-cianometiliden]-2-pirolidinon-5-karboksilat. V vseh primerih so reakcije potekle regiospecifično in stereoselektivno. Nastali so ustrezni spirolaktoni in spirolaktami z diastereoizomernimi presežki 20-34%.
V drugem delu smo pripravili tri nove (dimetilamino)metilidenske derivate tetramskih kislin, (3*E*,5*S*)-5-benzil-1-(*terc*-butoksikarbonil)-3-[(dimetilamino)metiliden]pirolidin-2,4-dion, (3*E*,5*S*)-5-benzil-1-benziloksikarbonil-3-[(dimetilamino)metiliden]pirolidin-2,4-dion in (3*E*)-1-[(*N*-benziloksikarbonil)glicil]-3-[(dimetilamino)metiliden]pirolidin-2,4-dion, ki smo jih uporabili kot izhodne spojine pri reakcijah z nukleofili in elektrofili. Opravili smo serijo pretvorb z različnimi amini, hidrazini in pirazolidin-3-oni ter izolirali produkte izmenjav dimetilaminske skupine. Z elektrofili smo pripravili 3-amino derivat tetramske kisline.
V zadnjem delu smo pripravili tri kiralne enamino ketone – (1*E*,4*S*)-4-(*terc*-butoksikarbonil)amino-1-dietilamino-5-fenil-pent-1-en-3-on, (1*E*,4*S*)-4-(benziloksikarbonil)amino-1-dietilamino-5-fenil-pent-1-en-3-on in benzil (4*S*,5*R*)-4-[(2*E*)-(3-*N*-metil-*N*-metoksiamino)akriloil]-2,2,5-trimetil-1,3-oksazolidin-3-karboksilat. Z reakcijami z različnimi dinukleofili in naknadnim odščitenjem aminske skupine smo jih pretvorili v kiralne amine in amino alkohole, ki so imeli vezano heterociklično enoto.

Stereoselective and Regioselective Synthesis and Transformations of some γ-Lactams and γ-Lactones

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In the first part of our research 1,3-dipolar cycloaddition reactions of diazomethane and nitril oxides to methyl (*S*)-3-[(*E*)-cyanomethylidene]-2-oxotetrahydrofurane-5-carboxylate and methyl (*S*)-1-*tert*-butoxycarbonyl-3-[(*E*)-cyanomethylidene]-2-pyrrolidinone-5-carboxylate were studied. All reactions proceeded regiospecifically and stereoselectively to give spirolactones and spirolactams as products with 20-34% d.e.
In the second part three new (dimethylamino)methylidene tetramic acid derivatives were prepared: (3*E*,5*S*)-5-benzyl-1-(*tert*-butoxycarbonyl)-3-[(dimethylamino)methylidene]pyrrolidine-2,4-dione, (3*E*,5*S*)-5-benzyl-1-benzyloxycarbonyl-3-[(dimethylamino)methylidene]pyrrolidine-2,4-dione and (3*E*)-1-[(*N*-benzyl oxycarbonyl)glycyl]-3-[(dimethylamino)methylidene]pyrrolidine-2,4-dione. These were used as starting compounds in the reactions with nucleophiles and electrophiles. A series of transformation using various amines, hydrazines and pyrazolidin-3-ones was performed to give the dimethylamino group substitution products. With electrophiles 3-amino tetramic acid derivative was prepared.
In the last part three chiral enamino ketones were prepared – (1*E*,4*S*)-4-(*tert*-butoxycarbonyl)amino-1-diethylamino-5-phenyl-pent-1-en-3-one, (1*E*,4*S*)-4-(benzyloxycarbonyl)amino-1-diethylamino-5-phenyl-pent-1-en-3-one and benzyl (4*S*,5*R*)-4-[(2*E*)-(3-*N*-methyl-*N*-methoxyamino)acryloyl]-2,2,5-trimethyl-1,3-oxazolidine-3-carboxylate, which were transformed into chiral amines and amino alcohols with an attached heterocyclic group *via* reactions with various dinucleophiles and subsequent deprotection of amino group.

Vloga zunajceličnega in znotrajceličnega katepsina B pri invaziji in ožiljanju tumorjev

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Lizosomska cisteinska proteaza katepsin B ima ključno vlogo pri razgradnji proteinov zunajceličnega matriksa, ki je povezana s procesi napredovanja raka, ni pa še pojasnjeno ali pri tem sodeluje zunajcelični ali znotrajcelični katepsin B. Delovanje katepsina B lahko sicer zavremo s specifičnimi inhibitorji, vendar do sedaj poznani inhibitorji ne omogočajo dovolj specifične inhibicije encima *in vivo*, predvidevali pa smo, da to lahko dosežemo z nevtralizacijskim monoklonskim protitelesom (Mab) 2A2, ki prepozna katepsin B in blokira njegovo encimsko delovanje. Na *in vitro* modelnih celičnih sistemih smo pokazali, da obstaja med humano *ras* transformirano epitelijsko celično linijo dojke MCF-10A neoT in endotelijsko celično linijo HUVEC razlika v razgradnji s fluoresceinom označenega proteinskega substrata. Z uporabo splošnih inhibitorjev cisteinskih proteaz, specifičnih inhibitorjev zunajceličnega in znotrajceličnega katepsina B in Mab 2A2 smo pokazali, da je za invazijo in ožiljanje in vitro potrebna aktivnost obeh frakcij katepsina B, vendar je aktivnost katepsina B bolj pomembna za invazijo tumorskih celic, kot za ožiljanje. In vitro se je monoklonsko protitelo dokazalo kot bolj učinkovit inhibitor katepsina B v primerjavi s sintetskimi inhibitorji, kar je verjetno posledica prehoda protitelesa v žive celice in inhibicije obeh frakcij katepsina B. Pomen katepsina B in drugih cisteinskih proteaz za invazijo in metastaziranje tumorskih celic smo potrdili tudi in vivo, na modelu tvorbe pljučnih metastaz pri miših, prav tako smo in vivo dokazali zaviralni učinek protitelesa 2A2. Naši rezultati so pokazali, da so pri zaviranju tumorske invazije bolj učinkoviti inhibitorji, ki delujejo na zunajcelični in znotrajcelični katepsin B in potrjujejo uporabnost nevtralizacijskega monoklonskega protitelesa 2A2 za zdravljenje raka.

The Role of Extracellular and Intracellular Cathepsin B in Tumour Invasion and Angiogenesis

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Lysosomal cysteine protease cathepsin B plays a central role in degradation of extracellular matrix proteins theirby facilitating tumour progression. It is not clear, whether extracellular or intracellular cathepsin B is involved in this process. Cathepsin B can be inhibited by specific inhibitors, however, the inhibitors known so far are not specific enough *in vivo*. Our hypothesis was, that improved specificity can be achieved by neutralising 2A2 monoclonal antibody (Mab), binding cathepsin B and inhibiting its enzymatic activity. Using model cell systems *in vitro*, we showed that *ras* transformed human breast epithelial cell line MCF-10A neoT and endothelial HUVEC cell line differed in degradation of fluorescein labelled protein substrate. With the use of general inhibitors of cysteine proteases, specific inhibitors of extracellular and intracellular cathepsin B and neutralising 2A2 Mab, we showed that cell invasion and angiogenesis *in vitro* depend on activity of both fractions of cathepsin B, and that proteolytic activity of cathepsin B is more important for invasion as it is for angiogenesis. Monoclonal antibody proved to be more effective inhibitor of cathepsin B *in vitro* than synthetic inhibitors, presumably as a result of antibody internalisation in living cells and inhibition of intracellular and extracellular cathepsin B. Importance of cathepsin B and other cysteine proteases for tumour cell invasion and metastasis was confirmed *in vivo*, on lung tumour metastasis model in mice, as was the inhibitory effect of neutralising 2A2 Mab. Our results show, that inhibitors acting on extracellular and intracellular cathepsin B are more effective in preventing tumour cell invasion, and, further more, demonstrate the applicability of neutralising 2A2 Mab in cancer treatment.

Mehanizem sproščanja histamina iz mastocitov podgane po aktivaciji z živčnim rastnim dejavnikom

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Mastociti so efektorske celice imunskega sistema, ki po aktivaciji sproščajo histamin in druge vnetne mediatorje. Poleg imunološke stimulacije povzroči sproščanje histamina iz mastocitov tudi endogeni živčni rastni dejavnik (NGF). V prvem delu naloge smo študirali vpliv zunajceličnih Na^+ in Ca^{2+} ionov na degranulacijo mastocitov po aktivaciji z NGF. Ugotovili smo, da v mediju s fiziološko koncentracijo Ca^{2+} ionov znižanje koncentracije Na^+ ionov zavira sproščanje histamina, medtem ko v mediju z nizko koncentracijo Ca^{2+} ionov odstranitev Na^+ ionov potencira sproščanje histamina iz mastocitov po aktivaciji z NGF. V drugem delu naloge smo s pomočjo inhibitorjev Na^+/Ca^{2+} in Na^+/H^+ izmenjevalca ugotovili, da koncentracija Na^+ in Ca^{2+} ionov v zunajceličnem mediju uravnava delovanje obeh izmenjevalcev. Na^+/Ca^{2+} izmenjevalec uravnava koncentracijo znotrajceličnih kalcijevih ionov, Na^+/H^+ izmenjevalec pa citosolni pH. Oba dejavnika aktivno vplivata na degranulacijo mastocitov. V tretjem sklopu naloge smo študirali katere encimske stopnje sodelujejo v prenosu signala, ki privede do degranulacije mastocitov po aktivaciji z NGF. Mehanizem aktivacije mastocitov z NGF smo primerjali z mehanizmom aktivacije, ki ga sproži stimulacija mastocitov z bazičnim sproščevalcem, spojino 48/80. Ugotovili smo, da se v procesu degranulacije mastocitov po aktivaciji z NGF aktivirajo encimi tirozin-kinaza, PI 3-kinaza, PLC in PKC. Aktivacijo istih encimskih stopenj, razen PI 3-kinaze, smo ugotovili tudi po aktivaciji mastocitov s spojino 48/80. Medtem ko inhibicija MAP-kinaz ni pokazala učinka na sproščanje histamina iz mastocitov niti po aktivaciji z NGF niti s spojino 48/80.

The Mechanism of Signal Transduction Pathway Involved in Nerve Growth Factor Induced Histamine Release from Rat Mast Cells

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Mast cells play a central role in allergic reactions. After activation they release histamine and a variety of inflammatory mediators. Beside immunologically induced mast cell activation, endogenous nerve growth factor (NGF) has been shown to induce mast cell degranulation. In the first group of our experiments, we studied the effect of extracellular Na^+ and Ca^{2+} ions on NGF induced histamine release from mast cells. We found that the removal of extracellular Na^+ ions reduced histamine release induced by NGF in the medium containing physiological concentration of Ca^{2+} ions. In contrast, in the medium containing low concentration of Ca^{2+} ions, the removal of Na^+ ions from the medium enhanced histamine release elicited by NGF. In the second group of experiments, we evaluated the effects of Na^+/Ca^{2+} and Na^+/H^+ exchanger inhibitors on NGF induced histamine release. Our results indicate, that changing the concentration of extracellular Na^+ and Ca^{2+} ions regulate the activity of Na^+/Ca^{2+} and Na^+/H^+ exchangers in mast cell membrane. Na^+/Ca^{2+} exchanger regulates the concentration of cytosolic calcium, whereas Na^+/H^+ exchanger plays an important role in intracellular pH regulation. Combined action of these exchange mechanisms serves to modify histamine release from mast cells. In the third part of our study, we investigated the signal transduction pathway involved in histamine release from mast cells provoked by NGF. The mechanism of secretion process induced by NGF was compared with compound 48/80 induced histamine release. We found that tyrosine kinase and PKC are involved in the signal transduction pathway responsible for either NGF or compound 48/80 induced histamine secretion from mast cells, whereas PI-3 kinase was activated only in NGF mediated degranulation process. In addition, degranulation of mast cells elicited either by NGF or by compound 48/80 was not affected by the inhibitors of MAP kinases.

Uporaba mikroskopa na atomsko silo za določanje morfoloških in energetskih lastnosti površin aktivnih in pomožnih sestavin zdravil

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Razlike v prosti površinski energiji trdnih snovi so lahko vzrok za tehnološke težave v farmacevtski industriji in za razlike v lastnostih končnih izdelkov. Uporaba mikroskopije na atomsko silo za določanje proste površinske energije je v farmaciji inovativna tehnika, ki smo jo vpeljali in preizkusili na sistemu polimernih filmov. Proučevali smo filme iz hidroksipropilmetilceluloze (HPMC), polivinilpirolidona (PVP), Eudragita® RS in Eudragita® RL. Vrednosti za prosto površinsko energijo oziroma kohezijsko delo, določene po Wujevi metodi na osnovi merjenja stičnega kota, smo primerjali z vrednostmi, ki smo jih pridobili z neposrednim merjenjem adhezijskih in kohezijskih sil s koloidno tehniko mikroskopije na atomsko silo. Ugotovili smo, da se izmerjeni adhezijska in kohezijska sila v primeru PVP z večanjem relativne vlažnosti (RV) zraka ob vzorcu večata, medtem ko izmerjene sile v ostalih sistemih ne kažejo izrazite odvisnosti od RV. Z uporabo obeh metod smo dobili enak vrstni red polimerov glede na naraščajočo vrednost površinske energije: RS = RL < HPMC < PVP, pri čemer so vrednosti tudi absolutno primerljive. Z mikroskopom na atomsko silo smo proučevali tudi proces rekristalizacije steklaste oblike felodipina in topografske značilnosti površine polimernih filmov.

The Use of Atomic Force Microscopy for Determination of Surface Morphology and Surface Energy of Active Ingredients and Excipients of Medicines

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The differences in the surface free energy of solids could lead to technological problems in pharmaceutical industry and to variable properties of the final product. The application of atomic force microscopy for determining surface free energy of pharmaceutical materials is an innovative technique, which was tested on polymer films. We were investigating the polymer films of hydroxypropylmethylcellulose (HPMC), polyvinylpirolydone (PVP), Eudragit® RS and Eudragit® RL. The values for surface free energy, determined by a contact angle measurement according to method of Wu, have been compared to the directly measured adhesive and cohesive forces using colloidal atomic force microscopy. We have found out that the adhesion and cohesion forces for PVP are very sensitive to relative humidity and that the forces in other systems are practically insensitive to the moisture. According both methods the polymers are sorted in similar order when their surface free energy is taken into account: RS = RL < HPMC < PVP. Even the numerical values are comparable. The atomic force microscopy was also used for investigation of the glassy felodipine recrystallization process and surface topography of polymer films.

Proučevanje strukture trdnih disperzij nifedipina z metodo temperaturno modulirane diferenčne dinamične kalorimetrije

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S proučevanjem taljenja binarnih sistemov nifedipin-manitol in nifedipin-PEG 4000 smo v prvem primeru ugotovili monotektično, v drugem pa evtektično razmerje. Priprava trdnih disperzij v vodi težko topnega nifedipina v kombinaciji z obema pomožnima snovema se je v smislu izboljšanja hitrosti raztapljanja izkazala ustreznejša v primerjavi s fizikalnimi zmesmi in učinkovino samo. Rezultati DSC, FTIR, XRPD ter mikroskopskih metod so potrdili razlog za ugodnejši potek raztapljanja: ustrezno dispergiranje učinkovine v sistemu kakor tudi izboljšan stik med učinkovino in pomožno snovjo. Prisotnost termodinamsko metastabilnih polimorfnih oblik pomožnih snovi je dodaten faktor, ki vodi v hitrejše raztapljanje.
V drugem delu disertacije smo proučevali vpliv parametrov temperaturnega programa modulirane DSC (MTDSC) na steklast prehod nifedipina. Ugotavljali smo tudi obseg motnje temperaturne modulacije kalorimetra z izdelavo Lissajousevih krivulj pri različnih kombinacijah amplitud, frekvenc (period) in povprečnih hitrostih segrevanja. Na osnovi ugotovitev smo za detekcijo steklastega prehoda s t.i. »heat-cool« postopkom izbrali periodo 60 s, amplitudo ± 1 °C in povprečno hitrost segrevanja 1 °C/min.
V nadaljevanju smo z izbranimi MTDSC parametri analizirali trdne disperzne sisteme in poleg predvidenih ali že ugotovljenih lastnosti dodatno ugotovili prisotnost sledov amorfne oblike nifedipina. Moduliran pristop je namreč omogočil analizo z izboljšano občutljivostjo in ločljivostjo v primerjavi s konvencionalno DSC metodo.

Investigation of Nifedipine Solid Dispersion Structure Using Modulated Temperature Differential Scanning Calorimetry

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The investigation of nifedipine-mannitol and nifedipine-PEG 4000 binary systems revealed monotectic and eutectic behaviour, respectively. The solid dispersion formation of poorly water soluble drug nifedipine in combination with both auxiliary substances resulted in improved dissolution rate compared to the physical mixtures or the pure drug. Results of DSC, FTIR, XRPD and microscopic methods confirmed the reason for favourable dissolution profile: appropriate dispersion of active ingredient in the system and improved contact between both components. The presence of thermodynamically metastable modifications of excipients can also be recognised as an additional factor for faster dissolution.
In the second part of the dissertation the influence of MTDSC parameters on nifedipine glass transition was examined. The extent of temperature modulation distortion was assessed by derivation of Lissajous curves using variety combinations of amplitude, period and underlying heating rate. Based on these findings, the period of 60 s, amplitude of ± 1 °C and 1 °C/min heating rate were selected to detect a glass transition with »heat-cool« procedure.
Further MTDSC analysis of solid dispersion systems indicated the presence of amorphous nifedipine traces. The modulated approach enabled the analysis of solid dispersions with improved sensitivity and resolution compared to the conventional DSC.

34. Krkine nagrade
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Cepitev molekule Bid kot možni mehanizem sprožitve apoptoze z lizosomskimi cisteinskimi proteazami

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Apoptoza je programirana oblika celične smrti, s katero organizem odstrani odvečne ali poškodovane celice. Glavni izvrševalci apoptoze so cisteinske proteaze kaspaze, ki se aktivirajo po eni od dveh poti aktivacije apoptoze: zunanji (ekstrinzični) ali mitohondrijski oz. notranji (ekstrinzični). Obe poti sta povezani s proapoptotsko molekulo iz družine Bcl-2 regulatorjev apoptoze, Bid.
Katepsini so lizosomske cisteinske proteaze, ki so pomembni za intralizosomsko razgradnje proteinov, sodelujejo pa tudi pri sprožitvi apoptoze. Ta je posledica prehajanja lizosomskih encimov v citosol zaradi zmanjšane stabilnosti lizosomskih membran, ki jo povzročijo lizosomotropne snovi, prosti kisikovi radikali, pojavlja pa se tudi pri staranju in različnih patoloških stanjih.
Z našim delom smo pokazali, da zmanjšanje stabilnosti lizosomskih membran povzroči aktivacijo (cepitev) molekule Bid s katepsini ter za apoptozo značilne morfološke in biokemijske spremembe celice. Z eksperimenti *in vitro* smo pokazali, da so za aktivacijo Bid odgovorne lizosomske cisteinske proteaze. Pokazali smo, da cepita katepsina B in K molekulo Bid na več mestih, vendar je glavno cepitveno mesto za Arg65, medtem ko je Arg71 glavno cepitveno mesto katepsina H. Katepsin X rekombinantnega Bid ne cepi. Pokazali smo tudi, da transfekcija celic 293 s skrajšanimi oblikami molekule Bid na mestih, kjer jo cepijo katepsini, sproži apoptozo, saj so transfektirane celice kazale za apoptozo značilne morfološke in biokemijske spremembe.
Ti rezultati kazejo, da lizosomske cisteinske proteaze lahko sprozijo apoptozo s cepitvijo molekule Bid.

Cleavage of Bid as a Possible Mechanism of Triggering Apoptosis by Lysosomal Cysteine Proteases

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Apoptosis or programmed cell death is used for removing excess or damaged cells from the organism. The main executors of apoptosis are cysteine proteases, caspases. There are two main apoptosis triggering pathways: extrinsic or intrinsic (mitochondrial) both of which lead to the activation of caspases. The two pathways are connected via the proapoptotic molecule Bid, a member of the family of Bcl-2-like regulators of apoptosis.
Cathepsins are lysosomal cysteine proteases, involved in intralysosomal degradation of the proteins. They are also involved in triggering apoptosis which is a consequence of decreased lysosomal membrane stability triggered by lysosomotropic agents, free reactive oxygen species, during aging or different pathological changes.
We have shown that decreased lysosomal stability causes activation (cleavage) of Bid by cathepsins, resulting in morphological and biochemical changes, typical of apoptosis. Using *in vitro* experiments we demonstrated that cleavage of Bid is caused by lysosomal cysteine proteases. Cathepsins B and K cleaved Bid at several sites, the major one being Arg65. Arg71 was the major cleavage site for cathepsin H while cathepsin X did not cleave recombinant Bid. We also demonstrated that transfection of 293 cells using mutant constructions of Bid, truncated on sites cleaved by different cathepsins, results in morphological and biochemical changes, characteristic of apoptosis.
These findings suggest the involvement of lysosomal cysteine proteases in the induction of apoptosis via Bid cleavage.

Uravnavanje izražanja kazeinskih genov pri konju (*Equus caballus*)

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Kazeini predstavljajo najpomembnejšo proteinsko frakcijo v mleku, kjer so glavni vir aminokislin za novorojence. Izražanje njihovih genov je kompleksno in ob indukciji z laktogenimi hormoni specifično poteka le v tkivu mlečne žleze. Podobnosti med človeškim in kobiljim mlekom nakazujejo na možnost uporabe kobiljega mleka kot bolj zdrave alternative kravjemu mleku. V tej raziskavi smo želeli natančneje preučiti mehanizme, ki uravnavajo izražanje kazeinskih genov pri konju, tako na ravni prepisovanja kot na ravni izrezovanja intronov.
V proksimalnem delu promotorjev β- in κ-kazeinskega gena pri konju smo določili elemente *cis*, ki pozitivno oziroma negativno uravnavajo izražanje obeh genov. Funkcionalnost vezavnih mest obeh promotorjev za aktivatorski faktor STAT5 smo preverili z metodo elektroforeznega zamika (EMSA). Inducibilnost β- in κ-kazeinskega promotorja smo preverili v poskusu transfekcije celic BME-UV 1/2 s konstrukti vektorja pGL3, ki so vsebovali različne dele kazeinskih promotorjev. κ-kazeinski promotor (1887 bp) je po dodatku laktogenih hormonov učinkoviteje aktiviral prepisovanje gena kot β-kazeinski promotor. Med oblikami β-kazeinskega promotorja je najučinkoviteje aktivirala prepisovanje gena kratka oblika (828 bp).
V α_{S1}- in β-kazeinskem genu smo dokazali po dva šibka eksona, ki se v procesu izrezovanja intronov alternativno vključujeta v zrelo mRNA: eksona 8 in 15 α_{S1}-kazeinskega gena in eksona 5 in 8 β-kazeinskega gena. Vzorec vključevanja šibkih eksonov za α_{S1}-kazein je bil pri vseh tridesetih živalih enak, pri β-kazeinu pa smo dokazali tri vzorce vključevanja obeh šibkih eksonov v zrelo mRNA. Različne vzorce smo povezali s polimorfizmoma, ki smo ju določili v β-kazeinskem intronu 1. Odvisnost vključevanja šibkih eksonov od variante na omenjenih mestih smo dokazali s poskusom izrezovanja intronov rekombinantega vektorja pSVEDATot v celični kulturi. Hkrati smo pokazali, da primarna struktura promotorja vpliva na izbiro mest izrezovanja v kotranskripcijskem procesu izrezovanja intronov.

Regulation of Equine Casein Gene Expression

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As a main protein fraction in milk, caseins are the most important source of amino acids for the suckling infant. Expression of casein genes is complex and tissue specific process. Due to high similarities between the human and mare milk's composition, mare's milk could be used as a healthier alternative to cow's milk. In this research we aimed to investigate the regulation of casein gene expression at the level of transcription as well as at the level of mRNA splicing. We searched for positive and negative *cis*-regulating elements in β- and κ-casein proximal promoter region. Using electro mobility shift assay (EMSA), we examined the functionality of transcription activator STAT5 binding sites in both promoters. We induced transcription of a reporter gene that was under control of β- and κ- casein promoters by lactogenic hormones and compared their efficiency of induction. Induction of gene expression by lactogenic hormones was more efficient under the control of the κ-casein promoter (1887 bp) compared to the β-casein promoter. The long β-casein promoter variant (1920 bp) induced weaker expression than the short one (828 bp).
Two alternatively spliced exons were found in the α_{S1}- (exons 8 and 15) as well as in the β-casein mRNA (exons 5 and 8). The analysis of α_{S1}-casein mRNA expression revealed the same pattern of inclusion of weak exons in the samples of all tested animals. In contrast, the β-casein mRNA showed three different splicing patterns of inclusion of weak exons. We found two polymorphisms in the β-casein intron 1, which were associated with different splicing patterns in all of the samples. Using *in vitro* splicing assay, we confirmed the association between the splicing patterns and polymorphic variants on the two sites in the β-casein intron 1. In addition, we demonstrated that the promoter primary structure affected splice site selection during the co-transcriptional splicing process.

Nevrotoksično in encimsko delovanje modrasovih fosfolipaz A_2

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Amoditoksini (Atx) so presinaptično nevrotoksične sekretorne fosfolipaze A_2 ($sPLA_2$) iz strupa modrasa, *Vipera ammodytes ammodytes*, amoditin I_2 ($AtnI_2$) pa je netoksična $sPLA_2$ iz istega strupa. Natančna vloga encimske aktivnosti pri še ne v celoti poznanem molekulskem mehanizmu presinaptične nevrotoksičnosti $sPLA_2$ je še vedno nejasna. S podrobno analizo encimskih lastnosti 16 kačjih naravnih in mutiranih $sPLA_2$ smo ugotovili, da so Atx zelo učinkoviti encimi pri delovanju tako na anionskih kot na nevtralnih membranskih površinah. Prisotnost anionskih fosfolipidov fosfatidilserina (PS) v nevtralnih membranskih površinah, bogatih s fosfatidilholinom, je močno vplivala na povišanje encimske aktivnosti kačjih $sPLA_2$, predvsem zaradi povišanja afinitete vezave na te membranske površine. Aromatski ostanki, predvsem triptofan, na določenih mestih stične vezavne površine Atx izredno pozitivno vplivajo na produktivno vezavo encima tako na nevtralne kot na anionske membranske površine in s tem na njegovo encimsko aktivnost. Glede na nizko afiniteto vezave in nizko encimsko aktivnost na veziklih, ki vsebujejo PS, netoksičen $AtnI_2$ izstopa iz skupine $sPLA_2$, uporabljenih v tej študiji. Atx so, kljub zelo specifičnemu in močnemu nevrotoksičnemu delovanju, v primerjavi s sesalskimi (netoksičnimi) $sPLA_2$ zelo učinkoviti encimi, kar je najverjetneje ključnega pomena v neki točno določeni stopnji procesa presinaptične nevrotoksičnosti.

Neurotoxic and Enzymatic Action of *Vipera Ammodytes Ammodytes* Phospholipases A_2

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Ammodytoxins (Atxs) are presynaptically neurotoxic secreted phospholipases A_2 ($sPLA_2$s) from venom of the long-nosed viper, *Vipera ammodytes ammodytes*, while ammodytin I_2 ($AtnI_2$) is a non-toxic $sPLA_2$ from the same venom. The exact role of enzymatic activity in the process of presynaptic neurotoxicity of $sPLA_2$s is still unknown. By carefully analysing the enzymatic properties of 16 wild-type and mutant snake venom $sPLA_2$s we showed that Atxs are very efficient enzymes when acting on anionic as well as on zwitterionic aggregated phospholipid substrates. The addition of anionic phosphatidylserine (PS) phospholipids to zwitterionic phosphatidylcholine vesicles induced an increase in the membrane-binding affinity leading to higher enzymatic activities of our enzymes. Certain aromatic IBS residues, especially tryptophan, have a very important role in productive binding of Atxs to both neutral and anionic membrane surfaces. $AtnI_2$ is unique among *V. a. ammodytes* $sPLA_2$s with its low membrane binding affinity and low enzymatic activity on the PS-containing vesicles. Despite the very potent and specific neurotoxic action of Atxs, they are very efficient enzymes in comparison to the whole range of mammalian (non-toxic) $sPLA_2$s, which is most probably of crucial importance in a specific, yet unidentified, step in the process of presynaptic neurotoxicity.

Simultaneous Analysis of Five Genetic Risk Factors in Polish Patients with Alzheimer's Disease

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As Alzheimer's disease (AD) is a complex disease, we decided to estimate how previously reported genetic polymorphisms interact to increase the risk for the disease. Five candidate genes were chosen: apolipoprotein E (APOE), alpha2-macroglobulin, cathepsin D, myeloperoxidase and nitric oxide synthase. Genotyping was performed in 100 cases of late-onset AD and 100 healthy controls.
We found a highly significant difference in APOE epsilon4 distribution between groups ($p.<0.005$). However, no evidence of association for other studied loci was found. Cumulative analysis of five genetic polymorphisms was performed, but it also failed to reveal any synergistic effect of candidate genes greater than that caused by APOE itself. Our results suggest that APOE epsilon4 allele is the only known genetic risk factor for late-onset, sporadic AD.

Correlative Study of Seizure Control, EEG Changes and Improvement in Cognitive Performance and Mood in Epileptic Patients Treated with Lamotrigine

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Background:
The current concept of successful antiepileptic drug treatment includes not only seizure control, but also favorable side-effects profile (the most concerned issue being the cognitive functions in epileptic patients). The aim of our study was to evaluate the neuropsychological effect of new generation antiepileptic drug Lamotrigine (LTG) trough tests on specific functions most affected in epilepsy. We also try to put more insight in the question whether these effects are correlated with seizure control and suppression of epileptiform activity or other mechanisms are involved.
Methods:
Sixty patients (age 12-58 years) taking LTG as mono and add-on therapy were investigated in this open, prospective, observational study. They belonged to two different syndrome groups: 1. Idiopathic epilepsies and 2. Symptomatic epilepsies with temporal lobe pathology. Routine and sometimes sleep-deprived EEG as well as neuropsychological assessment of cognitive functions (RAVLT and RCF for verbal learning and memory, recall and recognition, as well as visual/spatial memory) and mood (Profile index of emotions) was done baseline and 16 weeks after introduction of LTG.
Results:
68,4% of all patients experienced seizure reduction of > 50% and suppression of interictal epileptiform activity was found in 70% of patients.Neuropsychological studies showed improvement in cognitive performances (verbal and visual learning and memory) in 65% of patients, which parallel significantly both with EEG improvement and seizure control. Positive mood effects of LTG (in terms of its elevation and stabilization) were found in 76,6% of patients. In 21,7% of them this favourable psychotropic profile of the drug occurred despite unsatisfactory seizure control and/or lack of EEG improvement.
Conclusions:
Our results showed that positive properties of LTG on cognition and mood are well correlated with seizure control and EEG improvement. But, obviously this positive psychotropic effects do not depend exclusively on suppression of epileptiform activity, and other mechanisms are involved, especially in positively affected mood.

Pomen in prevalenca bakterijskih genov *cagA* in *iceA* pri otrocih iz Slovenije okuženih z *Helicobacter pylori*

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Številni geni bakterije *Helicobacter pylori*, kot *cagA*, *vacA* in *iceA*, so verjetno pomembni v patogenezi infekcije. V prospektivni študiji smo določili prevalenco in genetsko raznolikost *cagA* in *iceA* genov pri otrocih v Sloveniji ter povezanost okužbe z *Helicobacter pylori* z določenimi virulenčnimi geni in stopnjo bolezni prebavil. DNA smo izolirali iz 105 bioptov želodčne sluznice, ki smo jih odvzeli med endoskopijo zgornjih prebavil. S PCR specifičnimi primerji smo določili *cagA* in *iceA* status. Biopte so patohistološko opredelili glede na posodobljeni system sprejet v Sydneju. Gen *cagA* je bil prisoten v 68 (64,7%) primerih. Alel *iceA1* je bil prisoten v 62 (59%) od 105 izolatov in alel *iceA2* pri 33 (31,4%) bolnikih. Pet otrok (4,7%) je imelo prisotna oba alela in pri petih otrocih nismo uspeli določiti nobenega izmed alelov gena *iceA*. Dokazali smo statistično pomembno povezavo med *cagA* in *iceA* statusom (p=0,04 in p=0,004) ter stopnjo vnetja sluznice želodca. Za zaključek lahko rečemo, da je to študija o genotipih *Helicobacter pylori*, kjer je sodelovalo največje število otrok do sedaj na svetu in da smo dokazali da je okužba z bakterijo, ki ima *cagA* in *iceA1* gene, povezana z višjo stopnjo vnetja sluznice prebavil.

Prevalence and Role of *Helicobacter pylori cagA* and *iceA* Genes in Slovenian Pediatric Population

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Several *Helicobacter pylori* genes have been identified that probably play a role in the pathogenesis of infection, such as *cagA*, *vacA* and *iceA*. In our study we determined the prevalence and genetic diversity of *cagA* and *iceA* genes in Slovenian pediatric population. In addition the relationship between *cagA* and *iceA* genotypes and severity of gastroduodenal diseases in pediatric population was studied. DNA was isolated from 105 gastric biopsies previously used in rapid urease test. Biopsies were obtained from the same number of children. *H. pylori cagA* and *IceA* status of the isolates were determined by PCR using type specific primers. Biopsies were patohistologically evaluated according to Updated Sydney Classification. Among 105 children infected with *H. pylori cagA* gen was detected in 68 (64,7%) cases. Allelic genotype *iceA1* was identified in 62 (59,0%) and genotype *iceA2* in 33 (31,4%) patients. Five biopsy specimens (4,7%) contained both *iceA* allelic genotypes and in five cases we were not able to determine neither *iceA* alleles. Significant correlation between *cagA* and *iceA1* status (p=0.04 and p=0.004, respectively) and the severity of antral inflammation scored according to Updated Sydney classification were obtained. In conclusion, in the study, which according to published data included highest number of pediatric patients studied for *cagA* and *iceA* gene to date, we clearly demonstrate that infection with *cagA* and *iceA1 H. pylori* positive strains in children is associated with higher severity of antral inflammation.

Upravljanje znamk v razmerah internacionalizacije poslovanja organizacij

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Močne znamke igrajo pri uveljavljanju organizacij v svetovnem merilu pomembno vlogo, ki še narašča. Lahko predstavljajo tudi do 70% vrednosti organizacije. Kako pomembna oblika neotipljivega premoženja organizacije so znamke, pa so se organizacije v Sloveniji začele zavedati šele v zadnjih nekaj letih po osamosvojitvi, še bolj pa sedaj z vstopom v EU, ko smo na široko odprli vrata mednarodni konkurenci. Rezultat neustreznega upravljanja znamk v preteklosti je, da imamo Slovenci premalo mednarodno uveljavljenih znamk, kar nekateri radi opravičujejo z našo majhnostjo. Majhnost Slovenije sicer res predstavlja oteževalno okoliščino, vendar pa bi to dejstvo moralo še toliko bolj spodbujati organizacije k prodorni internacionalizaciji poslovanja. Navedeno nam je bilo osnovno vodilo za izbor teme magistrskega dela-tj. preučevanje kompleksnega procesa upravljanja znamk s posebnim poudarkom v razmerah internacionalizacije poslovanja organizacij. Prepričani smo, da s pričujočo raziskavo slovenskim organizacijam ponujamo osnove za kakovostno preobrazbo poslovanja na področju upravljanja znamk v naslednjih letih, ki jim bodo omogočale, če jih bodo upoštevale, uspešnejše konkuriranje znamkam svetovnega slovesa. Nedvomno je upravljanje znamk zahtevna in odgovorna naloga, strateškega pomena za organizacije, ki nikakor ne sme biti rezultat priložnostnih, kratkoročnih odločitev.

Brand Management Under Internationalized Business Operations

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Strong brands play an important role in establishing organizations on the international scale, and their role is even gaining in importance. They may account for even up to 70% of the organization value. Organizations in Slovenia have become aware of the importance of brands as a form of intangible assets only recently, in the last years after the independence, and even more so now with Slovenia entering the EU when the door has been wide open for international competition. The result of inadequate brand management in the past has led to the situation when the Slovenes have only a few internationally recognized brands, which some people relate to the country's smallness. This is indeed an aggravating circumstance, yet this fact should even stimulate the organizations to engage in penetrating business internationalization. The guidance for choosing our master's degree topic was to make a study of the complex process of brand management with special emphasis on the conditions of internationalized business operations. We are convinced that the current research might offer to the Slovene organizations certain bases for quality business transformation in the field of brand management in the years to come, which will enable the organizations, should they decide to respect them, to successfully compete with internationally renowned brands. Undoubtedly, brand management is a demanding and responsible task, of strategic importance for the organizations and it should in no way, come as a result of short-term random decisions.

Eksperimentalno načrtovanje procesov kristalizacije

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Kristalizacija je pomembna industrijska operacija, s katero iz proizvoda odstranimo nečistoče, zagotovimo njegovo pravilno obliko in velikost ter skrajšamo proizvodni proces. Za dosego določene kvalitete končnega proizvoda so potrebne obsežne laboratorijske raziskave, ki zahtevajo sistematičen pristop. V magistrskem delu predstavljamo podrobno načrtovanje procesov kristalizacije. Kot osnovo smo uporabili Taguchi-jevo metodo določanja nastavitev optimizacijskih parametrov procesa z minimalnim številom eksperimentov, ki jo je bilo za proučevanje kristalizacije potrebno modificirati. Po določitvi nivojev optimizacijskih parametrov kristalizacije in izbiri optimizacijskih kriterijev proizvoda, smo sistematično pristopili k načrtovanju procesa kristalizacije. Spreminjali smo glavne optimizacijske parametre kristalizacije in proučevali njihove medsebojne vplive. Iz standardne ortogonalne tabele in izbranih optimizacijskih parametrov smo določili število in zaporedje potrebnih eksperimentov. Rezultate eksperimentov smo obdelali s statistično metodo analizo variance, »ANOVA« ter določili ključne optimizacijske parametre in njihove optimalne vrednosti.
Načrtovanje eksperimentov kristalizacije smo izvedli na kristalizacijskem sistemu kalijev nitrat voda. Kot optimizacijske parametre smo obravnavali vrtilno frekvenco mešala, način in hitrost ohlajanja, cepljenje in druge, ki vplivajo na optimizacijske kriterije končnega proizvoda (velikost medianskega zrna, faktor enakomernosti zrnatosti, izkoristek, nasipno gostoto itd.). Kristalizacijo smo izvajali v avtomatskem laboratorijskem reakcijskem kalorimetru RC1 (Mettler Toledo), ki je omogočal vzdrževanje konstantnih parametrov procesa in zagotovil ponovljivost rezultatov.
Po določitvi optimalnih pogojev optimizacijski parametrov za izbran optimizacijski kriterij, smo v prvi fazi izvedli povečavo na 400 L in nato še na 4000 L kristalizator. Pri povečavi smo zagotovili enake ohlajevalne čase in enak prenos snovi, kot v laboratorijskem merilu. Za uspešno povečavo smo določili ustrezno vrtilno frekvenco mešala in pretok hladilnega sredstva skozi plašč kristalizatorja.

Experimental Design of Crystallization Processes

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Crystallization has become one of the most important unit operations in chemical industries. The need to reduce the time from product discovery to market introduction is an inherent concern. In order to achieve the prescribed product quality characteristics, the process of engineering experimentation has to be optimized. Therefore, an experimental design method for crystallization processes is presented in this work. Initially, the standardized Taguchi method was used to plan a minimum number of experiments. After identifying the working levels of the optimization parameters and the optimization characteristics of the product under consideration, the method has been successfully applied to crystallization processes. The simultaneous variations of main crystallization parameters and their interactions were investigated using the orthogonal array technique. A statistical analysis of variances, »ANOVA« including product optimization characteristic was performed. After developing some special criteria, which depend on performance objectives, the optimal levels of the optimization parameters were determined.
Crystallization of KNO_3 with defined product optimization characteristics (particle size, uniformity grain coefficient etc.) was used to obtain optimization parameters. The effects of rotational frequency of the stirrer, cooling rate, seeding and other parameters on optimization characteristics were studied. In order to keep the selected parameters constant during the experiment and to ensure reproduction of the entire experiment the automated reaction calorimeter RC1 was used.

Vpliv tehnoloških parametrov na nastanek in lastnosti nanosuspenzij

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Vse večje število na novo sintetiziranih zdravilnih učinkovin je težko topnih. Biološka uporabnost takšnih učinkovin je posledično zelo majhna in pogosto ne doseže terapevtskih vrednosti. Težko topne učinkovine je s tradicionalnimi tehnološkimi pristopi težko oblikovati v ustrezne farmacevtske oblike, kar je razlog, da jih pogosto zavržejo že zgodaj v razvoju. Sodoben in obetajoč pristop za povečanje biološke uporabnosti težko topnih učinkovin predstavlja oblikovanje nanosuspenzij. Nanosuspenzije so definirane kot disperzije delcev učinkovine nanometriskih velikosti (<1000 nm) v tekočem mediju. Tehnologije za oblikovanje nanosuspenzij, ki se trenutno uporabljajo, vključujejo obarjanje, mletje mikronizirane učinkovine in homogeniziranje pod visokim tlakom.
Namen raziskovalne naloge je bil pripraviti nanosuspenzije s tehnologijo razredčevanja emulzij tipa o/v v katerih notranjo fazo predstavlja topilo, ki se delno meša z vodo, ter ugotoviti vpliv tehnoloških parametrov na lastnosti izdelanih nanosuspenzij. Za modelno učinkoviono smo izbrali ketoprofen. Na nastanek nanosuspenzij imajo odločilni vpliv farmacevtsko tehnološki parametri. Le-ti morajo biti strogo nadzorovani, da dobimo nanosuspenzije z želenimi lastnostmi. Z izbrano sestavo vstopnih komponent in z ustreznimi tehnološkimi parametri nam je uspelo pripraviti in ovrednotiti nanosuspenzije s povprečno velikostjo delcev 80 nm in polidisperznim indeksom 0,2.

Influence of Technological Parameters on Formation and Properties of Nanosuspensions

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An increasing number of newly synthesized drugs are poorly soluble. Consequently the bioavailability of such drugs is poor and very often below the therapeutic level. They are difficult to develop as effective drug products using traditional formulation techniques and are frequently abandoned early in discovery. An alternative and promising approach to improve bioavailability of poorly soluble drugs is the formulation of nanosuspensions. A nanosuspension is defined as a dispersion of nanosized drug particles (<1000 nm) in liquidmedium. Production technologies currently used are precipitation, pearl milling of micronized drug and high pressure homogenization.
The aim of this research work was to investigate the feasibility of preparing drug nanosuspensions by dilution of o/w emulsions containing partly water-miscible solvents and to study the influence of production parameters on the characteristics of prepared nanosuspensions. Ketoprofen was used as a model drug. All formulation parameters should be under control to get nanosuspension with desired properties. Using optimized formulations and homogenization parameters, nanoparticles with average particle size of 80 nm and polydispersity index of 0,2 were prepared.

Informatizacija upravljanja kakovosti v podjetju Krka

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Podjetja se morajo hitro prilagajati trenutnim razmeram na trgu, zato je potrebno nenehno prilagajati ter optimizirati s tem povezane poslovne procese, hkrati pa zadržati kolikor se da natančen nadzor nad poslovanjem za kakovostno sprejemanje nadaljnjih strateških odločitev. Poslovni informacijski sistemi igrajo kot orodje za učinkovito spremljanje poti do zastavljenih ciljev na tem področju ključno vlogo. V delu sem analizirala uvajanje integriranega informacijskega sistema SAP v Krki na področju upravljanja kakovosti. Temeljni cilj je bil prikazati prehojeno pot od odločitve po uvedbi sistema do poteka uvajanja ter pokazati procesni pristop, ki se je uporabil na tej poti.
Zaključene ugotovitve so namenjene povzetku analize uvajanja sistema, skladno z rezultati, kateri so bili že doseženi skozi proces uvajanja. Hkrati so prikazani nadaljnji koraki uvajanja. Novi informacijski sistem naj bi zagotavljal boljšo organiziranost, več kontrole in manjšo možnost napake v poslovnih procesih. Zagotoviti čim boljši in čim kakovostnejši izdelek, ki mu bo potrošnik zaupal, pa je ključno vodilo vsakega podjetja

Information System of Quality Management in Krka

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Companies must adjust to market conditions very quickly and that is the reason why they have to accommodate and optimize business processes linked with this. But at the same time they have to keep very precise control over operation for taking quality strategic decisions. Business information systems as an effective tool have the key role in achieving set aims. In my Master's degree I have analyzed implementing the integrative information system SAP in Krka in the field of quality management. The main aim was to explain a decision why to implement such a system and show the process of implementing the SAP system.
Results which were obtained during implementation are described in conclusion. At the same time the next steps of the project are shown. The new information system assures better organizing, more control and less possibility for mistakes in the process. The best quality product which the customer trust the most is the primary motive of every company.

Vodenje energetskih tokov v farmacevtski industriji

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Vodenje energetskih tokov v farmacevtskem podjetju je zahtevna in kompleksna naloga. Zato smo vzpostavili informacijski center energetike s pomočjo katerega nadziramo obratovanje energetskih sistemov, vodimo energetske tokove ter ugotavljamo energetsko učinkovitost sistemov. S tem smo za pet zmanjšali število zaposlenih v energetski oskrbi, kar predstavlja 11,4%. S pomočjo analiz, ki nam jih tak sistem omogoča, smo izvedli projekt izrabe odpadne toplote v kompresorski postaji in dosegli letni prihranek 319.000 Sm^3 zemeljskega plina in zmanjšali letno emisijo CO_2 za 592t. Kot eno izmed možnih opcij nadgradnje informacijskega sistema smo razvili vpeljavo trajnostnega poročila za podjetje.

Energy Flow Management in Pharmaceutical Industry

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Energy flow management in pharmaceutical company is demanding and complex task. For this reason the energy control center was implemented which helps us to control running of energy systems and to manage energy flows in the factory as well as to finding out energy systems efficiency. With this approach the number of employees was reduced for five persons what means 11,4% in energy supply department. Such control system enables to make analyses on basis of which the project of energy conservation in compressor station was carried out. Savings of 319.000 Sm^3 of natural gas and reduction of CO_2 emissions for 592 tons were achieved. As one of potential options of upgrading the energy control system implementation of sustainable report into company was developed.

Vrednotenje naložb: realne opcije pri investicijskem odločanju in strateškem načrtovanju

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Temeljna hipoteza tega dela je, da v današnjem globalnem in dinamičnem ekonomskem okolju klasične metode za vrednotenje investicij ne zadoščajo, če želijo podjetja izboljšati investicijske odločitve in tudi tako dosegati strateške konkurenčne prednosti. To pa se lahko doseže z uporabo metode realnih opcij. Hipotezo testiram in dokazujem teoretično, kakor tudi aplikativno, saj je namen magistrskega dela podati konceptualno podlago podjetjem za razvoj modelov vrednotenja investicijskih projektov, torej za uporabo metode realnih opcij v praksi.
Večina tržne vrednosti podjetja ponavadi ne izhaja iz njegovih obstoječih dejavnosti, temveč iz možnosti podjetja za dodatno rast in razvoj. To še posebej velja za podjetja v hitro rastočih panogah, kot so elektronika, telekomunikacije, farmacija in biotehnologija. Uspešna podjetja v takšnih panogah nenehno vlagajo sredstva v ustvarjanje novih opcij, ki jih potem selektivno izkoriščajo. Opcijska vrednost določene naložbe izhaja iz njene prilagodljivosti. Da bi izbrali najboljšo naložbo, je potrebno prilagodljivost s pomočjo opcijske analize pravilno ovrednotiti. Metoda realnih opcij deluje, ker pomaga managerjem vključevati priložnosti, ki jih imajo, v načrtovanje in upravljanje strateških odločitev.
Ker imajo realne opcije lahko znatno vrednost, je pomembno, da se klasične metode naložbene analize nadgradijo tudi z metodami, ki upoštevajo realne opcije, skrite v posameznih naložbenih odločitvah.

Capital Investment Valuation: Real Options in Capital Budgeting Decision-Making and Strategic Planning

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The main hypothesis of this work is that in today's global and dynamic economic environment classical methods for capital investment valuation do not suffice if companies want to improve investment decisions so as to reach strategic competitive advantages. This can be achieved by using a real option method. I have tested and proved the hypothesis theoretically as well as practically, because the purpose of my master thesis is to give companies a conceptual basis to develop models for investment projects valuation, thus to put a real option method into practice.
Most of the company's value usually doesn't arise from its current activities but from its chances for additional growth and development. This is particularly true for companies in fast growing branches like electronics, telecommunications, pharmacy and biotechnology. Successful companies in such branches continuously invest in new options creation, which they afterwards selectively execute. An options value of an investment is derived from its flexibility. For choosing the best investment it is important to correctly value the flexibility with assistance of option analysis. The real options approach works because it helps managers to plan and manage strategic investments with opportunities they have.
Real options can have a substantial value, so it is important to upgrade the classical methods of investment analysis with the methods that consider real options hidden in the individual investment decisions.

Določevanje vsebnosti cikorne kisline v posušenem soku škrlatne ehinaceje (*Echinacea purpurea*) s tekočinsko kromatografijo visoke ločljivosti in kapilarno elektroforezo

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Škrlatna ehinaceja (*Echinacea purpurea* Moench) je ena redkih rastlin, ki stimulira delovanje nespecifičnega imunskega sistema. Vsebuje številne spojine, najpomembnejše pa so polisaharidi, derivati kavne kisline (predvsem cikorna kislina) in alkamidi. Ker učinkovina ni znana, je potrebno za opredelitev kakovosti droge ali soka izbrati ustrezno označevalno snov, ki odraža delovanje. Kot primerno smo izbrali cikorno kislino, kajti raziskave kažejo, da ima imunostimulativno delovanje, verjetno se absorbira v zadostni količini in je občutljiva na razgradnjo. V nalogi smo preizkusili dve analizni metodi: HPLC in CE, primerni za določevanje vsebnosti cikorne kisline, ki smo ju našli v literaturi. Pri privzemu obeh metod se je izkazalo, da je HPLC metoda uporabna in ne zahteva večjih posegov. CE metoda ni bila optimalna za izbrano označevalno snov, zato smo posegli v vse kritične faze analize, jih spremenili in tako je nastala nova metoda za določevanje vsebnosti cikorne kisline v soku *E. purpurea*. Ker stroški analiz pri rutinski kontroli niso zanemarljivi, smo pri obeh metodah uporabili interni standard, ki je cenovno najugodnejši, pa tudi v drogi ali soku ga ni mogoče najti, to je gentizinsko kislino. Obe optimirani metodi smo tudi validirali in primerjali njune validacijske parametre. Pri primerjavi smo ugotovili, da je za rutinsko določevanje vsebnosti primernejša HPLC metoda, ki je ponovljivejša, natančnejša in točnejša kot CE metoda. Kljub temu pa je tudi nova CE metoda primerna za rutinsko analizo, vendar z upoštevanjem omejitev glede na dobljene validacijske parametre.

Determination of Cichoric Acid Content in Dried Pressjuice of Purple Coneflower (*Echinacea purpurea*) with High Performance Liquid Chromatography and Capillary Electrophoresis

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Purple coneflower (*Echinacea purpurea* Moench) is one of the rare plants that stimulate non-specific immune system. It contains multiple substances amongst which the most important are polysaccharides, caffeic acid derivatives (especially cichoric acid) and alkamides. Since the active substance is not known, appropriate marker, that reflects the effect, has to be chosen in order to determinate juice or drug quality. As an appropriate marker in this work, cichoric acid was used, because research shows that it has immune stimulatory affects, it is most probably absorbed in adequate quantity and it is susceptible to degradation. In this work two analytical methods: HPLC and CE were tested. These methods are, according to the literature, suitable for cichoric acid assay. With adoption of both methods, HPLC method proved to be useful and it demanded no major modifications, however CE method was not optimal for the chosen marker. All the critical phases of CE analysis were altered, which led to a new CE method for cichoric acid content determination in *E. purpurea* pressed juice. Internal standard-gentisic acid was used for two reasons: first, costs of routine control are not negligible and gentisic acid proved to be cost wise most appropriate, second gentisic acid can not be found in the drug or the pressed juice. Both optimised methods were validated and its parameters of validation were compared. Comparisment showed that for routine content determination HPLC method is better, because of its repeatability, accuracy, and precision. On the contrary, new CE method is useful for routine analysis, with limitations considering acquired validation parameters.

Znotrajcelični inhibitorji cisteinskih proteaz dermatofita *Microsporum canis*

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V nalogi prvi opisujemo inhibitorno aktivnost proti cisteinskim proteazam v citosolnem ekstraktu *Microsporum canis (M. canis)*. Proteinske inhibitorje smo izolirali z afinitetno kromatografijo. Iz rezultatov poliakrilamidne gelske elektroforeze z natrijevim dodecilsulfatom sklepamo, da je relativna molekulska masa izoliranega proteina okrog 28 kDa, in da je inhibitor homodimer. Z imunokemijsko detekcijo na membrani smo ugotovili, da imajo vse testirane, naravno okužene mačke v serumu protitelesa proti proteinskemu inhibitorju, vključno s kontrolno, na *M. canis* negativno, skupino živali. Nizkomolekularne inhibitorje smo ločevali s tekočinsko kromatografijo visoke ločljivosti na reverzni fazi. Izolirani nizkomolekularni inhibitor je stabilen v širokem temperaturnem (50–90 ºC) in pH (2–12) območju. Oba izolirana inhibitorja zavirata proteolizno aktivnost katepsina B, nizkomolekularni inhibitor pa tudi katepsina L. Katepsina H ne inhibirata, kakor tudi ne serinske proteaze tripsina. Možno je, da imata izolirana inhibitorja endogene regulatorne funkcije, saj je znano, da *M. canis* med drugim sintetizira tudi cisteinske proteaze. Pri rasti v tekočem gojišču, kjer posnema parazitski način rasti, tvori tudi veliko količino inhibitorjev, iz česar bi lahko domnevali, da bi omenjeni inhibitorji lahko sodelovali pri patogenezi mikrosporoze z zaustavljenjem hidrolizne aktivnosti cisteinskih proteaz v koži gostitelja ali z vplivom na njegov imunski odziv.

Intracellular Inhibitors of Cysteine Proteases from the Dermatophyte *Microsporum canis*

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We describe, for the first time, the inhibitory activity against cysteine proteases in the cytosolic extract of *Microsporum canis (M. canis)*. The protein inhibitor was isolated by affinity chromatography. The results of SDS-polyacrylamide gel electrophoresis showed that the protein inhibitor has a molecular mass of about 28 kDa, indicating homodimer composition. Western immunoblotting showed that all tested, naturally infected cats produced antibodies against the protein inhibitor, including the *M. canis* negative control group of cats. Low molecular mass cysteine protease inhibitors were separated with reverse phase high performance liquid chromatography. The isolated low molecular mass inhibitor is stable in a wide temperature range (50-90 ºC) and a wide pH range (pH 2-12). Both isolated inhibitors from *M. canis* impair the hydrolytic activity of cathepsin B, the low molecular mass inhibitor also that of cathepsin L. Cathepsin H as well as serine protease trypsin are not inhibited by the isolated inhibitors. It could be possible that isolated inhibitors have endogenous regulatory functions; it is known *M. canis* produces cysteine proteases, too. Growing in the liquid medium, and thus resembling the parasitic life phase, the dermatophyte expresses large amounts of inhibitors, indicating they could be involved in the pathogenesis of microsporosis by inhibiting the proteolytic activity of cysteine proteases in the host skin or by interfering with the host immune response.

Ugled in uglednostni kapital v farmacevtski industriji v Sloveniji

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V okviru proučevanja ugleda in uglednostnega kapitala v farmacevtski industriji v Sloveniji sem preverjala tri hipoteze, in sicer, da se podjetja v Sloveniji ne ukvarjajo sistematično z upravljanjem ugleda, da imajo podjetja z uspešnim upravljanjem komunikacij v povprečju boljši ugled in da sta ugled in uglednostni kapital v farmacevtski industriji nad povprečjem ostalih panog. Proučevanje pomena in vloge komunikacijskih aktivnosti je temeljilo na Grunigovem modelu odličnosti, ugleda pa na Fombrunovem modelu uglednostnega količnika in izračunu uglednostnega kapitala. V delu je najprej predstavljeno poslovno komuniciranje in vloga odnosov z javnostmi v sodobni družbi, sledi prikaz neopredmetenega premoženja, v osrednjem sklopu tega dela pa je z različnih vidikov predstavljen ugled in uglednostni kapital. Praktični del naloge temelji na prikazu ugleda in uglednostnega kapitala farmacevtskega podjetja Krka. Poleg predstavitve farmacevtske panoge in podjetja, je natančneje prikazano še komuniciranje in odnosi z javnostmi v tem podjetju ter temelji in merjenje ugleda. Percepcijsko merjenje ugleda Krke je prikazano skozi rezultate različnih raziskav, bistvo celotnega dela pa je v predstavitvi rezultatov, dobljenih na podlagi anketiranja treh skupin javnosti (splošna, poslovna, strokovna). V raziskavi sem s pomočjo izračuna uglednostnih količnikov ugotovila, da ima Krka v očeh različnih javnosti nadpovprečno dober ugled, da je Krkin uglednostni količnik primerljiv z uglednostmi količniki podjetij po vsem svetu in tudi v različnih časovnih obdobjih. Uglednostni kapital Krke raste iz leta v leto in je bil v primerjavi z ostalimi najuglednejšimi podjetji v Sloveniji v letu 2003 največji.

The Reputation and the Reputational Capital in Pharmaceutical Industry in Slovenia

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When researching the reputation and the reputational capital in pharmaceutical industry in Slovenia, I have analyzed three hypotheses, which are the following: companies in Slovenia do not systematically deal with reputation management, companies with successful communications management have generally a better reputation, and reputation and reputational capital in the pharmaceutical industry are above average when compared to other branches. The analysis of the importance and role of communication activities was based on the Grunig model of excellence, while the analysis of reputation was based on the Fombrun model of reputational quotient and on calculation of the reputational capital. The paper presents business communication and the role of public relations in modern society, followed by a review of intangibles and presentation of the reputation and reputational capital from different perspectives. In addition to presenting the pharmaceutical branches and the company itself, it also presents communication and public relations within this company, as well as the foundations and measuring of reputation. The perceptive measuring of Krka's reputation is shown through results of different researches and the main point is to present the results based on a poll made for three public groups (common public, medical experts, business public). This can be verified by results of different researchers, which have always placed Krka in the top few of the most respected companies. In my paper I have also calculated the reputational quotient and came to conclusion that Krka has an above-average positive reputation and that its reputational quotient is comparable to those of most respected companies around the world in different time periods. The calculation of reputational capital of Krka also shows the growth of reputational capital and was ranked highest, in comparison with other companies in 2003.

Importance of Genotyping and Phenotyping in Bioequivalence of Drugs

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The research of bioequivalence of drugs is the basic method for estimation of quality of generics and allows to give the conclusion about the quality of drugs using lesser number of volunteers, than in clinical trials. The standard criteria of inclusion of volunteers do not allow to predict individual features of biotransformation's system. Individual pharmacokinetic parameters in result of researches of some drugs can differ more than in 20% volunteers. An estimation of the factors influencing metabolism of drugs provide more adequate representation of results. Taking into account large percent of prevalence of genetic polymorphism of CYP 2D6 in a population the basic technique allows preliminary forecasting of drug's plasma concentration depending on speed of metabolism in a liver, is genotyping of isoenzymes with polymerase chain reaction (PCR). Genotyping of various sites of a gene CYP 2D6 has 95% efficiency of revealing individuals with poor metabolism. Substratum specificity allows to develop methods of phenotyping CYP 3A4. The enzyme activity is defined on pharmacokinetic of its specific substratum, named "marker" substratum, by measurement of drug concentration and concentration of its metabolite in plasma. The offered test is based on measurement of concentration of metabolite lidocaine –MEGX and use it as a marker of activity CYP3A4. Thus, the application of genotyping and phenotyping in bioequivalence researches allows to reduce disorder individual meanings and to predict adverse pharmacological "answers" at study of drugs.

Računalniški vid v farmacevtski industriji

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Računalniški vid je postal splošno priznan način vizualne kontrole kakovosti in pogosto celo kritičen del proizvodnega procesa, še posebno v farmacevtski industriji. Vedno ostrejše zahteve po kakovosti in nižjih proizvodnih stroških v povezavi s količinsko ogromnimi proizvodnimi kapacitetami, so postavile sisteme na osnovi računalniškega vida v vlogo vitalnih orodij, s katerimi je mogoče zadostiti zahtevam in doseči zastavljene cilje v masovni proizvodnji farmacevtskih izdelkov.
Magistrsko delo obravnava uporabo računalniškega oziroma umetnega vida v farmacevtski industriji. V drugem poglavju so opisane osnovne lastnosti računalniškega vida in prikazani postopki zajema ter razgradnje slike, ki predstavljata osnovni fazi pri delovanju vsakega sistema na osnovi računalniškega vida. V tretjem poglavju se magistrsko delo tematsko dotakne regulatornih predpisov, ki veljajo pri uporabi računalniških sistemov v farmacevtski proizvodnji, ter na tem področju najbolj znanih priporočil, zbranih pod imenom 21-CFR-11, ki jih izdaja ameriška FDA (Food and Drug Administration). V četrtem poglavju je prikazan splošen pregled uporabe računalniškega vida v proizvodnji farmacevtskih izdelkov, ki deluje na osnovi vidnega dela svetlobe. Predstavljene so osnove in glavni pristopi pri uporabi računalniškega vida ter opisane najbolj pogoste aplikacije v farmacevtski proizvodnji. V naslednjem poglavju je predstavljena uporaba NIR-a (Near Infrared Spectroscopy), oziroma bližnje infrardeče spektroskopije v analitske namene v farmacevtski proizvodnji. NIR uporablja človeku nevidni del spektra elektromagnetnega valovanja. Glavna uporabna vrednost bližnje infrardeče spektroskopije je dejstvo, da za razliko od računalniškega vida na osnovi vidnega dela svetlobe, ki ga uporabljamo za kontrolo vizualnih lastnosti izdelkov, z uporabo NIR-a lahko sklepamo na kemijsko sestavo nadzorovanih proizvodov. V šestem poglavju so podrobneje opisani sistemi za vizualno pregledovanje posameznih tablet, izdelana pa je tudi primerjava obstoječih sistemov različnih proizvajalcev.

Computer Vision in Pharmaceutical Industry

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Computer vision systems have become well established in quality inspection and are often crucial parts of manufacturing processes. This is especially evident for mass production in pharmaceutical industry in which computer vision systems plays a vital role due to higher and higher demands for quality inspection improvement and manufacturing costs reduction.
This Master thesis deals with the applications of computer vision systems in pharmaceutical industry. Basic properties of computer vision systems and procedures for image acquisition and segmentation, which are the most important parts of any computer vision system, are described in the second chapter. In the third chapter, relevant regulation for computer vision systems in pharmaceutical industry is addressed. The most relevant regulation in this area is the recommendation 21-CFR-11 (Part 11 of Title 21 of Code of Federal Regulations) that was issued by the FDA (Food and Drug Administration). A general overview of computer vision systems, based on the visible part of light, and most commonly used in pharmaceutical industry is given in the fourth chapter. In the chapter that follows, the use of NIR (Near Infrared Spectroscopy) for analytical purposes is described. NIR systems work in the invisible part of the electromagnetic spectrum and, therefore, enable the detection of the chemical structure of the analysed materials. In the last chapter, the existing computer vision systems for inspection of individual tablets are described in detail and inter-system comparison is provided.

Izolacija aktivne farmacevtske učinkovine iz sintezne mešanice s solventno ekstrakcijo

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Za načrtovanje ekstrakcijskih procesov je ključnega pomena poznavanje faznega ravnotežja tekoče-tekoče. Ravnotežne podatke tekoče-tekoče običajno potrebujemo v obliki porazdelitvenih koeficientov za vsako komponento sistema.
V tem delu obravnavamo izolacijo aktivne farmacevtske učinkovine (API) iz reakcijske mešanice po uparevanju ekstrakcijskega topila z uporabo solventne ekstrakcije. Raziskan je bil vpliv termodinamskih in kinetskih parametrov, ki so nujni za načrtovanje industrijskega procesa. Narejen je bil izbor topila in preverjeni so bili različni načini izvedbe ekstrakcije. Zelo pomemben kriterij, ki je determiniral izbor primernega ekstrakcijskega topila za proizvodni proces, je zahteva, da ima sol aktivne učinkovine zelo nizko topnost v ekstrakcijskem topilu, kar je pogojeno s samim postopkom preciptacije soli. To je seveda v nasprotju z osnovnim kriterijem za izbiro topila. Zaradi nizkega porazdelitvenega koeficienta v izbranem topilu je bila potrebna prisotnost anorganske soli v lužnici, ki vstopa v sistem solventne ekstrakcije z namenom zvišanja topnosti topljenca v organski fazi. Zaradi tega dejstva je bil uporabljena solventna ekstrakcija z izsoljevanjem (salting out solvent extraction). Raziskano je bilo fazno ravnotežje (psevdo)ternarnega sistema: napajalna vodna faza, aktivna farmacevtska učinkovina (kot topljenec) in izbrano organsko topilo; pri tem nečistote in prisotnost soli niso bile upoštevane. Raziskan je bil tudi vpliv ostalih pomembnih procesnih parametrov, kot so na primer temperatura, pH, vsebnost anorganske soli in razmerja med ekstrakcijskim topilom in vstopno lužnico. Sistem je bil raziskan na laboratorijskem in pilotnem nivoju in predlagani so pogoji in rešitve za izvedbo industrijskega procesa.

Recovery of Active Pharmaceutical Ingredient from Reaction Mixture with Solvent Extraction

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A good description of liquid-liquid equilibrium is important to design extraction separation processes. Equilibrium data are normally needed in the form of distribution coefficients for each individual component under consideration.
In the thesis investigation of ternary system for liquid-liquid extraction of an active pharmaceutical ingredient from reaction mixture after evaporation of reaction solvent has been presented. The system was investigated from standpoint of thermodynamic and kinetic parameters, that are essential for industrial process development. Solvent selection was made and all basic types of extraction procesess were assesed.
One very important criteria for selection of suitable solvent for the production process is that the final product (an API salt), has low solubility in common extraction solvents. This is however in contrary with basic selection kriteria for extraction solvents. Because of very low partition coefficients of solute in selected solvent, presence of inorganic salt is needed in the raw liquor to increase the solubility of solute. For that reason salting out solvent extraction (SOE) was applied. In additon for typical reaction mixture after evaporating of solvent, liquid-liquid equlibria was studied. Influence of some important process parameters such as temperature, pH, content of anorganic salt and solvent feed ratio were discussed. The system was checked in laboratory and pilot scale, so that optimal conditions for industrial process were proposed.

Sponzoriranje kot element trženjskega komuniciranja in ugled podjetja

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Sponzoriranje se zavoljo spreminjajočega se družbenega okolja enakovredno postavlja ob bok drugim orodjem trženjskega komuniciranja. To potrjuje vse večje število sponzorjev in iz leta v leto višji sponzorski zneski, kar posledično pomeni, da se sponzoriranju kot učinkovitemu elementu trženjskega komuniciranja namenja veliko pozornosti. Razlog za uveljavljanje in sprejemanje sponzoriranja kot vse bolj pomembne metode trženjskega komuniciranja v zadnjih desetletjih naj bi bil v iskanju novih in stroškovno učinkovitih načinov komuniciranja s korporativnimi javnostmi. V pričujočem delu obravnavam sponzoriranje kot element trženjsko komunikacijskega spleta, pri čemer sem prvi del namenila pojasnjevanju samega pojma sponzoriranja, procesa njegovega razvijanja znotraj komunikacijske strategije podjetja ter pomena njegovega strateškega razvijanja za podjetja. Ugled je pogosto cilj sponzorskega delovanja podjetij in tako v nadaljevanju sistematično pojasnjujem pojem ugleda z vidika njegovega razvijanja in pomena za podjetje. V empiričnem delu predstavljam izsledke dveh lastnih raziskav. Na podlagi izsledkov prve, opravljene med naključno izbranimi prebivalci Dolenjske, sem preverila zastavljene raziskovalne hipoteze o povezanosti med sponzorskimi dejavnostmi podjetja, prepoznavnostjo sponzorskih dejavnosti, pogostostjo spremljanja sponzoriranih dogodkov in različnimi dimenzijami ugleda. Drugo raziskavo pa sem opravila v sodelovanju s podjetji, ki sponzorirajo na področju Dolenjske in Bele krajine. Analizirala sem njihove sponzorske strategije ter uspešnost le-teh v povezavi z izsledki prve raziskave.

Sponsorship as Element of Marketing Communications and Company Reputation

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Due to the changes in social environment, sponsorship is being equally treated as other marketing communication tools. This fact has been confirmed by the growing number of sponsors and increasing sponsorship expenditures each year. Consequently sponsorship as an effective element of marketing communication is given a lot of attention. The reason for bringing sponsorship forward and for its reception in the last two decades could be explained by the need for search of new and cost effective methods of communication with the corporate public. In presented work I have introduced sponsorship as an integral part of marketing communications mix. The explanations of the conception and description of the process of sponsorship management within the communication strategy of the company are described in the first part, as well as the importance of its strategic planning for the company. Reputation is often a goal of sponsorship activities and therefore I have given a systematic explanation of reputation, its management, and its importance for the company. Empirical part consists of the results of two private researches. On the basis of the results of the first one, done among the inhabitants of Dolenjska, I tested the research hypothesis concerning the connection between sponsorship activities of the companies, acquaintance and frequency of attendance to the sponsoring activities, and different dimensions of the reputation. Second research was done in co-operation with the companies that are sponsoring in Dolenjska and Bela krajina. I have analysed their sponsorship strategies and their effectiveness in the connection to the results of the first mentioned research.

Predlog načrta za program promocije zdravja na delovnem mestu v podjetju Kolektor, d.o.o.

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Promocija zdravja na delovnem mestu predstavlja skupen trud delavcev, delodajalcev in širšega družbenega okolja za izboljšanje zdravja delovne populacije. Vključuje preventivne in protektivne aktivnosti, ki vzdržujejo in krepijo fizične in psihične sposobnosti ter motivacijo delavcev. Cilji promocije zdravja se uresničujejo z izboljševanjem organizacije dela in delovnega okolja, aktivnim sodelovanjem v procesu in spodbujanjem osebne rasti zaposlenih.
Cilj specialistične naloge je bil pripraviti program promocije zdravja za zaposlene v podjetju Kolektor. V ta namen je bila napravljena analiza bolniškega staleža za petletno obdobje 1998 do 2002, ki je pokazala, da se je odstotek bolniškega staleža v omenjenem obdobju pomembno zniževal in da so bile poglavitni vzrok zanj poškodbe, poleg teh pa še bolezni dihal, mišično skeletnega sistema in nosečnost pri ženskah. Izvedena je bila tudi anketa o delovnem mestu, zdravstvenem stanju in življenjskem slogu, ki je pokazala visoko stopnjo pripadnosti podjetju in relativno dobro zdravstveno stanje zaposlenih.
Na osnovi podatkov o trenutnem zdravstvenem stanju zaposlenih so bile določene prioritete, ki naj bi jim program promocije zdravja sledil in obsegajo dvigovanje varnostne kulture delavcev oz. odpravo poškodb pri delu, zniževanje števila poškodb zunaj dela, krepitev splošne obrambne sposobnosti organizma in preprečevanje nalezljivih bolezni dihal, izboljševanje stanja lokomotornega aparata, promocijo kulture gibanja, uvajanje uravnotežene prehrane v vsakodnevni jedilnik zaposlenih in nenazadnje celovito politiko obvladovanja bolezni odvisnosti.

Workplace Health Promotion Programme Plan Proposal for Kolektor

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Workplace health promotion represents mutual effort of workers, employers and surrounding social environment whose aim is to improve health of the working population, to reduce number of early retirement cases and excessive sick-leave, to prevent injuries at work and to prevent occupational diseases or diseases related to work. It includes preventive and protective actions that maintain and strengthen workers' physical and psychical capability and also raise their work motivation. The aims of workplace health promotion are implemented by improving work organization and direct working environment, by active participation of employees and by promoting their personal growth.
The aim of this paper work is to create a workplace health promotion programme for the employees of Kolektor. In this purpose the analysis of sick-leave for five-years (1998 to 2002) was done. Injuries have been recognised to be the most important reason for workers' absence. Other important reasons are also diseases of respiratory tract, skeletal system and pregnancy (women). A questionnaire on workplace, health status and lifestyle was performed that showed high level of workers loyalty to the company, and a relatively high level of actual employees' health status.
Based on these data the priorities were determined that health promotion programme needs to follow. These include raising the safety culture of workers and diminishing the number of cases of work (and other) injures, strengthening the defence capability of an organism (immunity) and preventing infectious diseases, improving health status of skeletal system, physical activity and balanced nutrition promotion and last but not least complex nicotine, drug and alcohol addiction politics in the company.

Oblikovanje strategije raziskav in razvoja kot funkcijske strategije v generičnem podjetju farmacevtske panoge

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V generični farmacevtski industriji je razvojna dejavnost ključna gonilna sila in je osnova za perspektivno možnost posameznega podjetja. Izziv raziskav in razvoja je raziskovanje tehnologij, ki vodijo podjetje v nove posle, ne da bi pri tem prezrle tehnologijo, ki je potrebna za ohranjanje glavnega področja delovanja.
Kot ključni element uspešnosti raziskovalno-razvojne dejavnosti generičnega farmacevtskega podjetja se vedno bolj kaže sposobnost izbire in kombiniranja posameznih razvojnih projektov kot tudi razporejanja kapacitet v okviru njihove realizacije. Kot pomoč pri tem delu predlagamo uporabo pristopa 360°, prirejenega za področje generičnega farmacevtskega podjetja. Ta s pomočjo različnih ocenjevanj identificirane projekte vrednoti glede na: ekonomsko uspešnost, strateško pomembnost za podjetje, možnost razvoja, čas in možnost uporabe ostalih razvojnih virov.
Po našem mnenju predstavlja metoda 360° orodje, ki z uporabo analitičnih prijemov pri razvrščanju med posameznimi raziskovalno-razvojnimi projekti zagotavlja najprimernejši pristop pri odločitvi o strategiji raziskav in razvoja.

Establishing a Research and Development Strategy as a Functional Strategy in a Generic Pharmaceutical Company

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In a generic pharmaceutical company, research means a key driving force and a cornerstone of a promising development of individual company. A special challenge for research and development is investigation of new technologies, which would lead the company to new business operations and at the same time preserve the established ones, necessary to maintain the core business activity.
The key element of a successful research and development of a generic pharmaceutical company is the ability to choose and combine individual development projects as well as to efficiently distribute the capacities for their implementation. In view of this, we propose to use the approach 360°, which has been adjusted to the needs of a generic pharmaceutical company. Using different assessment scales this approach evaluates the identified projects according to: economic efficiency, strategic importance for a company, development possibilities, time frame and possibility to use other development sources.
We believe that the method 360° is a tool which ensures the most suitable approach to a choice of research and development strategy by using the analytical methods for distribution of individual research and development projects.

Farmakološke študije kompleksnih antagonistov: vpliv karvedilola na kontraktilnost atrija budre

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V tem raziskovalnem delu smo želeli kot farmakološko orodje opredeliti karvedilol, zdravilo, ki se že vrsto let uporablja za zdravljenje hipertenzije in srčnega popuščanja. Karvedilol je na izoliranem desnem atriju budre, ki je relativno neobčutljiva na delovanje noradrenalina, deloval kot zelo močan, nekompetitiven antagonist adrenergičnih receptorjev β (K_b = 0,1 nM) ter inhibitor kalcijevih in kalijevih kanalov (K_b = 30μM), vendar ne od ATP-odvisnega podtipa kalijevih kanalov. Kljub temu, da so bili pogoji med poskusom ravnotežni in da sam preparat ne vpliva na frekvenco kontrakcije, ne moremo izključiti možnih eksperimentalnih napak. Te napake in različni mehanizmi delovanja karvedilola na izoliranem desnem atriju bi lahko bili vzrok prikritega kompetitivnega antagonizma na adrenergičnih receptorjih β. Zaključimo lahko, da učinkovita zdravila niso tudi odlična farmakološka orodja. Zaradi blokade ionskih kanalov in sočasnega zaviralnega učinka na adrenergične receptorje β bi bilo mogoče smiselno preveriti možnost uporabe karvedilola pri zdravljenju motenj srčnega ritma.

Pharmacological Studies of Complex Antagonists: The Influence of Carvedilol on Guinea Pig Atria Contractility

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In this research we wanted to evaluate carvedilol as a pharmacological tool. Carvedilol is an effective and safe drug, used as an antihypertensive and heart failure drug. We wanted to analyse the action of carvedilol on adrenergic receptors β, calcium and potassium channels in isolated guinea pig right atrium. In spite of the fact that guinea pig is rather insensitive to noradrenergic action, carvedilol was proved to be a strong non-competitive β-adrenergic antagonist (K_b = 0,1 nM), as well as calcium and potassium channel blocker (K_b = 30 μM), but not of $K_{(ATP)}$ channel subtype. The experimental errors cannot be ruled out in spite of the fact that experiments were carried out in equilibrium and the kinetic properties of the isolated organ did not affect its action throughout the experiment. These, and multiple mechanisms of action of carvedilol on isolated guinea pig atrium can contribute to the observation that the antagonism of carvedilol to adrenergic receptors β is not competitive. It can be concluded that effective drugs are not equieffective pharmacological tools, but due to simultaneous β-adrenergic receptor blockade and calcium and potassium channel inhibition it may be reasonable to test the use of carvedilol as an antiarrhythmic drug.

Izbira metode za določitev toksičnega vpliva fototransformiranih presnovkov fluorantena na testno bakterijo *Pseudomonas putida*

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Fluoranten sodi v razred policikličnih aromatskih ogljikovodikov, ki so vseprisotni okoljski polutanti, in je znan kancerogen in genotoksični agens. Presnovki razgradnje fluorantena, ki nastajajo v procesu biodegradacije s sevom *Pasteurella sp.* IFA, so v okolju izpostavljeni različnim procesom, v katerih se njihova toksičnost lahko spremeni. Z raziskavo smo skušali ugotoviti, v kolikšni meri se spreminja toksičnost presnovkov kot posledica fototransformacije. V ta namen smo snovi v 2-urnem intervalu obsevali s sončno svetlobo, toksičnost nastalih produktov pa testirali kot inhibicijo rasti na testni bakteriji *Pseudomonas putida*. Ugotovitve so bile presenetljive, saj je v kratkem času prišlo do večjih sprememb v smeri zmanjšanja toksičnosti. V primeru benzojske kisline in fenilocetne kisline pride do zmanjšanja inhibicije rasti za 15%, v primeru adipinske kisline do 30%, v primeru ftalne kisline pa do 45% zmanjšanja inhibicije rasti. IC_{50} vrednosti za te spojine pred obsevanjem so med 87 in 108 mg/l, po obsevanju pa se gibljejo med 106 in 156 mg/l, kar je znak zmanjšanja toksičnosti. Izmed metod, s katerimi lahko vpliv fototransformacije določimo, smo izbrali metodo merjenja absorbance z dodatkom rastnega indikatorja TTC (2,3,5-trifeniltetrazolijev klorid), ki se je izkazala kot bolj občutljivo, predvsem pa bolj natančno spremlja metabolno dogajanje v celici.

Selection of Method for Determination of Toxic Effects of Phototransformed Metabolites of Fluoranthene on Test Bacteria *Pseudomonas putida*

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Fluoranthene is among the most ubiquitous polycyclic aromatic hydrocarbons in nature and is known for its genotoxic and cancerogenic effect. Metabolites of fluoranthene are exposed to different processes in environment where their toxicity might be changed. Our research tried to establish modification of toxicity due to phototransformation. For this purpose metabolites of fluoranthene were irradiated with sun light for two hours and toxicity was tested as growth inhibiton on test bacteria *Pseudomonas putida*. Findings were surprising because in very short period of time larger modification in reduction of toxicity was noticed. Diminishing of growth inhibiton for benzoic acid and phenylacetic acid was 15%, for adipic acid 30% and for phtalic acid 45%. IC_{50} values for these compounds before irradiaton were between 87 and 108 mg/l, after irradiaton between 106 and 156 mg/l which indicates reduction of toxicity. Among methods suitable for evaluation of phototransformation effect method with additon of growth indicator TTC (2,3,5-triphenyl-tetrazolium chloride) was chosen. It demonstrated higher sensitivity and accuracy in determination of metabolic occurrence in the cell.

Vpliv časa zadrževanja pelet in tablet v umetnem želodčnem soku na sproščanje paracetamola

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Zadrževanje farmacevtske oblike v želodcu je eden od dejavnikov, ki lahko vpliva na hitrost sproščanja oziroma absorpcije učinkovine, zato smo z modificiranim testom raztapljanja poskušali ovrednotiti vpliv zadrževanja pelet z upočasnjenim in tablet s hitrim sproščanjem v umetnem želodčnem soku na hitrost raztapljanja paracetamola. Na koncu smo naredili še *in vitro*/*in vivo* korelacijo.
Test sproščanja smo izvajali na sistemu peristaltične črpalke in magnetnega mešala s kontroliranimi obrati in temperaturo in spreminjali čas zadrževanja farmacevtske oblike v želodčnem soku. Ker ne vemo natančno, kako hitro prehajajo pelete skozi dvanajstnik, smo z *in vitro* testiranjem proučevali tudi vpliv hitrosti spremembe pH medija iz kislega v nevtralno na hitrost sproščanja učinkovine.
Čas zadrževanja tablet v umetnem želodčnem soku ni statistično signifikantno vplival na hitrost sproščanja paracetamola in tudi profili sproščanja paracetamola iz pelet so si bili med seboj podobni in le na nekaterih mestih statistično signifikantno različni. Poleg tega nismo zasledili statistično signifikantnega vpliva hitrosti spreminjanja pH medija na hitrost sproščanja paracetamola iz pelet. Če te podatke prenesemo *in vivo* lahko sklepamo, da naj praznjenje želodca in hitrost prehoda skozi dvanajstnik ne bi imela velikega vpliva na *in vivo* hitrost sproščanja paracetamola.
In vitro/*in vivo* korelacija za pelete nam je dala premico z visokim koeficientom korelacije, koeficient korelacije pa je bil nekoliko nižji v primeru tablet. V obeh primerih je prišlo do zamika, kar je bilo pričakovati, saj je po literaturnih podatkih absorpcija paracetamola iz želodca zanemarljiva. Naklona korelacij za pelete in tablete nista bila enaka in sta odstopala od vrednosti 1. Iz dobljenih korelacij lahko sklepamo, da je pri testu sproščanja nek parameter odstopal od dejanskih *in vivo* pogojev.

Effect of Pelets' and Tablets' Residence Time in Artificial Gastric Juice on Release of Paracetamol

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The residence time of a dosage form in the stomach is one of the parameters that can influence dissolution rate and drug absorption. Therefore, modified dissolution test was employed to study the influence of residence time of the sustained release pellets and immediate release tablets in artificial gastric juice on the dissolution rate of paracetamol. *In vitro*/*in vivo* correlation was also established.
Our dissolution test based on the system of peristaltic pump and magnetic stirrer, which maintained the temperature and the stirring rate of the medium, with different residence time of the dosage form in the gastric juice. Since we do not know the passage rate of the pellets through the duodenum, we tried to evaluate the influence of the rate of pH increase on the dissolution rate of the drug from the pellets with *in vitro* testing.
Tablets' residence time in gastric juice did not have a statistically significant influence on the dissolution rate of paracetamol. Also, the release profiles of paracetamol from pellets were similar and only in a few points statistically significant. No statistically significant influence of the rate of pH increase from acid to neutral medium on the dissolution rate of the paracetamol from the pellets was observed. Thus, gastric emptying and transit time through the duodendum are not expected to have a great influence on the *in vivo* dissolution of paracetamol.
In vitro/*in vivo* correlation for pellets was linear, with high coefficient of correlation. On the other hand, the coefficient of correlation for tablets was not that high. There was a significant lag in both cases, as it was expected, while, according to literature data, the absorption from the stomach is negligible. The slopes of the curves were not equal and were both different from value 1 as well. Obtained *in vitro*/*in vivo* correlations lead us to the conclusion, that there was a parameter that differed from the *in vivo* conditions.

Trženje storitev za tuje trge v zdravilišču Šmarješke Toplice

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V nalogi se osredotočam na zdraviliški in wellness turizem kot njegov pomemben segment. Razvoj Zdravilišča Šmarješke Toplice sem umestila v predstavitev razvoja področja v Sloveniji. Slovenski zdraviliški turizem lahko doseže pomembne konkurenčne prednosti, zato so strateški cilji turizma na tem področju v Sloveniji izredno pomembni. Ponudnikom je pomembno osvojiti različne ciljne skupine gostov: tako tistih, ki obiskujejo zdravilišče iz zdravstvenih rekonvalescentnih razlogov, kot drugih, ki iščejo tovrstne storitve iz preventivnih razlogov. Zlasti slednjih je vse več; wellness programi pa pomemben tovrstni konkurenčni produkt ne samo za goste iz Slovenije, ampak vse bolj tudi za goste iz tujine. Prav k slednji ciljni skupini je osredotočena naloga. Pri analizi turistov iz različnih držav sem ugotovila, da imajo le-ti različne želje in navade dopustovanja. V nalogi ugotavljam, da če želi Zdravilišče Šmarješke Toplice dosegati nadaljnji porast prihodka od gostov iz Italije, Avstrije in Nemčije, potem mora prilagoditi svoje programe posameznim ciljnim skupinam.

Marketing Services for Foreign Markets in Šmarješke Toplice Spa Resort

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In the assignment I am focusing on the significant segment of the spa and wellness tourism. I intervened the growth of Šmarješke Toplice spa resort to present the growth of this field in Slovenia. Slovenian spa tourism can achieve significant competitive advantages. That`s why the strategic goal of tourism in this region of Slovenia is very important. It is important that the offering companies capture different target group of guests: the ones that are visiting the spa for health convalescence reasons and the ones that are visiting the spa for preventative reasons. The latter guests are visiting more and more often; wellness programme is an essential competitive product, for the Slovenian guests and even more often for the foreign guests. This assignment is focused on the latter target group. By analyzing guests from different countries I found that their wishes and vacation rituals were different. I find in the assignment that if Šmarješke Toplice Spa resort wishes to achieve further financial growth from the guests from Italy, Austria and Germany then they will have to adjust their programs for separate target groups.

Mapiranje epitopov na kokošji IgY molekuli z monoklonskimi protitelesi

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Kokošja protitelesa IgY se v nekaterih lastnostih razlikujejo od sesalskih IgG, zato v različnih imunoloških testih vedno pogosteje nadomeščajo mišja in kunčja protitelesa. Pri proizvodnji poliklonskih, monoklonskih in rekombinantnih IgY protiteles je potrebno testiranje prisotnosti teh molekul v vmesnih stopnjah proizvodnje, zadnji korak pa je izolacija molekul IgY oz. njenih podenot. Pripravili smo monoklonska protitelesa proti molekuli IgY (mAb proti IgY), karakterizirali mesta njihove vezave na IgY molekuli ter določili navzkrižne reakcije mAb proti kokošjim IgY z IgY drugih vrst ptic. Pridobili smo tri tipe mAb proti IgY: mAb 4E4, ki prepoznavajo samo lahke verige kokošjih IgY; mAb 3C10, ki prepoznavajo poleg lahke verige kokošjih IgY še puranje, fazanje in vrabčje IgY molekule in mAb 2F10, ki reagirajo s težko verigo kokošjih IgY in prepoznavajo še molekule IgY puranov, pavov in fazanov. Vsa pridobljena mAb prepoznavajo neglikolizirano determinanto na molekuli IgY. Pripravili smo imunoafinitetno kolono (CNBr) in jo uspešno uporabili za izolacijo kokošjih IgY. Ker poznamo epitope na IgY molekuli, s katerimi reagirajo mAb proti IgY, lahko to metodo uporabimo za izolacijo katerekoli podenote IgY (Fab, Fc, lahke ali težke verige) in tako izvedemo izolacijo bodisi poliklonskih IgY iz rumenjaka ali seruma imunizirane kokoši, monoklonskih IgY iz gojitvenega medija ali rekombinantno pridobljenih podenot molekule IgY. Sama mAb proti kokošjim IgY so potencialno uporabna za odkrivanje okužb v intenzivni reji perutnine ter idealno orodje za izolacijo IgY molekul, skupaj s kokošjimi IgY pa tvorijo idealno orodje v humani diagnostiki.

Epitope Mapping of Chicken IgY Molecule Using Monoclonal Antibodies

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Due to their specific nature chicken antibodies IgY are more and more popular substitute to mammal antibodies used in immuno-diagnostic tests. In the production of polyclonal, monoclonal and recombinant IgY antibodies their detection and testing in intermediate phases is needed. Finally, isolation of IgY molecules or its subunits is required. We produced monoclonal antibodies against chicken IgY molecule (mAb anti IgY), described their binding domains on the IgY molecule and determined cross reactions of mAbs anti IgY with IgY from different avian species. Produced mAbs were of three types: mAb 4E4, which recognize only the light chain of chicken IgY; mAb 3C10, which recognize the light chain of chicken IgY as well as turkey, pheasant and sparrow IgY molecules; and mAb 2F10, which react with the heavy chain of chicken IgY and recognize also turkey, peafowl and pheasant IgY molecules. All acquired mAb recognize the nonglycosilated determinant on the IgY molecule. We prepared an immunoaffinity column (CNBr) and used it for the chicken IgY isolation successfully. Since binding epitopes are known any IgY subunit (Fab, Fc, light chain, heavy chain) from any source (egg yolk, serum, culture supernatant) can be isolated using appropriate mAb anti IgY coupled in the column. Our mAbs can potentially be useful for the detection of poultry infection in intensive poultry breeding while together with IgY they can effectively replace mammal antibodies in human diagnostics.

Vpliv genskih polimorfizmov v presnovni poti folata na tveganje za akutno limfatično levkemijo pri otrocih

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Znotrajcelična koncentracija folata je ključni dejavnik za normalno rast celic. V presnovno pot folata so vključeni encimi 5,10-metilentetrahidrofolat reduktaza (MTHFR), timidilat sintaza (TS), metionin sintaza (MS) in metionin sintaze reduktaza (MTRR), za katere je znano, da polimorfizmi v pripadajočih genih vplivajo na njihovo aktivnost. Spremenjena aktivnost teh encimov lahko vpliva na sintezo, popravljanje in metilacijo DNK, kar lahko predstavlja dejavnik tveganja za akutno limfatično levkemijo (ALL).
Namen našega dela je bil z metodo genotipizacije določiti pogostnost polimorfnih alelov in genotipov ter njihove kombinacije genov za MTHFR, TS, MS in MTRR pri kontrolni skupini ter pri bolnikih z ALL. S statistično obdelavo podatkov smo ugotovili, da se razlike v frekvencah polimorfizma MTHFR 677 v skupini bolnikov z ALL ter kontrolni skupini študentov statistično značilno razlikujejo (P = 0,045) ter da polimorfizem MTHFR 677TT 2,9-krat zniža tveganje za ALL in je tako zaščitni dejavnik za ALL. Razlike v frekvencah polimorfizmov MTHFR A1298C, MS A2756G, MTRR A66G in TS med obema skupinama pa niso bile statistično značilne, zato smo sklepali, da te polimorfizmi niso dejavnik tveganja za ALL. V bodoče bi z genotipizacijo polimorfnih genov v presnovni poti folata lahko bolnikom z ALL prilagodili odmerek zdravila glede na njihov genotip, zmanjšali toksične učinke zdravljenja ter izboljšali preživetje in kakovost življenja.

Genetic Polimorphisms in Folate Metabolism and the Risk for Acute Lymphoblastic Leukemia in Children

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Tetrahydrofolate (THF) is necessary for normal cell growth. Enzymes that are involved in THF metabolism are 5, 10-methylenetetrahydrofolate reductase (MTHFR), thymidilate synthase (TS), methionine synthase (MS) and methionine synthase reductase (MTRR). Polymorphisms in their corresponding genes alter enzymatic activity and influence DNA synthesis, methylation and repair, thus increasing the risk of acute lymphoblastic leukemia (ALL).
The allele frequency, genotypes and their combinations for MTHFR, TS, MS and MTRR genes in 66 patients with ALL and 104 healthy controls were determined with genotypization method. Statistical analysis have confirmed that there were significant differences in frequencies of polymorphic genotypes MTHFR 677 between controls and patients with ALL (P = 0,045), but there were no differences observed in polymorphism frequencies of MTHFR A1298C, MS A2756G, MTRR A66G and TS between both groups. Our results suggest that MTHFR 677 polymorphism 2,9-fold decreased the risk of ALL and was therefore a protective factor of ALL. Other MTHFR A1298C, MS A2756G, MTRR A66G and TS polymorphisms or their combinations were not associated with the risk of ALL. In future, molecular genetic methods could contribute to individualization of ALL treatment.

Vpliv koncentracije vodne faze na stabilnost zgoščenih O/V ME pri različnih temperaturah

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V raziskovalni nalogi smo preučevali zgoščene mikroemulzije (ME) olja v vodi (O/V). ME so bile sestavljene iz naslednjih, netoksičnih komponent: izopropil miristata, destilirane vode, Tween 80 in benzil alkohola. Kot gostilo zunanje vodne faze, smo uporabili ksantan, mikrobni polisaharid, ki v vodi tvori šibko gelsko strukturo. Zgoščene O/V ME so izkazovale podobno reološko obnašanje kot vodne raztopine gostila enake koncentracije, torej viskoelastične lastnosti, visoko viskoznost in strižno odvisno tokovno obnašanje.
Za proučevanje stabilnosti zgoščene O/V ME v odvisnosti od količine vodne faze smo pripravili tri vzorce z različno sestavo in enako koncentracijo gostila. Zgoščena O/V ME z največjo vsebnostjo oljne, dispergirane faze je po enem tednu razpadla v dvofazni sistem. Zato smo se nadaljnjih raziskavah osredotočili na preostali dve zgoščeni O/V ME. Stabilnost zgoščenih O/V ME smo proučevali na osnovi določanja reoloških lastnosti proučevanih vzorcev. Meritve smo izvajali z reometrom z nastavljivo strižno napetostjo. Vpliv temperature na stabilnost vzorcev smo preverili pri 6 °C, 20 °C in 32 °C. Vpliv strižne sile na tokovne lastnosti vzorcev, in posledično stabilnost vzorcev pod vplivom striga, smo določali pri destruktivnih strižnih pogojih. Viskoelastične lastnosti vzorcev smo določali z oscilacijskimi testi pri ne-destruktivnih strižnih pogojih.
Ugotovili smo, da je zgoščena mikroemulzija z večjo vsebnostjo vodne faze, torej tudi višjo vsebnostjo gostila (ksantan), stabilnejša. Le ta je izkazovala manjšo temperaturno odvisnost viskoznosti in manj izrazito strižno in časovno odvisno obnašanje. Pri obeh tipih mikroemulzij je pri povišani temperaturi (32 °C) prišlo do tvorbe novih tridimenzionalnih struktur pod vplivom destruktivnih strižnih sil zaradi dodatnih interakcij emulgator-polimer (gostilo).

The Influence of Water Phase Concentration on Stability of Thickened O/W ME at Different Temperatures

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The research deals with the stability of thickened oil in water microemulsions, (o/w ME). The non-toxic components of examined o/w ME, were: isopropyl myristat, distilled water, Tween 80 and benzyl alcohol. As a thickener for the water (outer) phase was used xanthan, microbial polysaccharide. The polymer in a water-soluble ordered form (in aqueous solutions) generally exhibits high viscosity at low shear rates and elastic-solid behaviour under non-destructive shear conditions. Thickened microemulsions exhibited the similar rheological behaviour, to that observed for aqueous xanthan solution of the same polymer concentration.
To examine the influence of water content on the stability of o/w ME three samples of different composition and the same thickener concentration, were prepared. One week after the preparation thickened o/w ME composed of the highest amount of oil (dispersed phase) exhibited a phase separation, and therefore was not used for further investigations. The stability of thickened o/w ME was examined by rheological characterization of studied samples. Measurements at 6 °C, 20 °C in 32 °C were performed by using controlled stress rheometer. The effect of shear stress on the flow properties, and consequently the stability of the samples under shear was studied under destructive shear conditions. Viscoelastic properties were determined under non-destructive conditions of oscillatory shear.
It was found that o/w ME composed of higher water content and, consequently, with higher amount of the thickener, behaved more stable. It exhibited less pronounced temperature effects on rheological properties and less pronounced time dependent effects on shear flow. Under destructive shear conditions, independently of water content, an additional formation of three-dimensional network structures, arising from surfactant-thickener interactions, was observed at higher temperature (32 °C).

Razvoj kromatografskih metod sklopljenih z masno spektrometrijo za določevanje alprazolama v plazmi

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V delu je predstavljen razvoj analizne metode za določevanje alprazolama (ALP) v plazmi. Alprazolam je zdravilna učinkovina, ki se uporablja za zdravljenje depresije in odpravljanje zaskrbljenosti. Cilj dela je bil razviti dovolj občutljivo, ponovljivo in linearno metodo za kvantitativno določevanje alprazolama v plazmi za namene farmakokinetičnih študij. Odločila smo se za analizno tehniko GC-MS, ki je tehnika z dobro resolucijo in občutljivostjo in dodatno zanesljivo identifikacijo spojine, to je z masnim spektrom spojine. Razvito metodo na GC/MS smo poskušali prenesti še na LC-MS/MS z namenom višje občutljivosti (nižje LOQ) in specifičnosti. Razvoj analizne metode vključuje tudi pripravo plazemskih vzorcev z ekstrakcijo T-T in SPE. Uspešni sta bili obe vrsti ekstrakcije. Pri ekstrakciji T-T smo uporabili organsko topilo butilklorid v mediju pH 9. SPE ekstrakcijo smo razvili na ekstrakcijski koloni Strata-X; aktivne komponente smo eluirali z acetonitrilom. GC/MS metoda je linearna v koncentracijskem območju od 1 do 100 ng ALP/ml plazme z LOQ 1 ng ALP/ml plazme ter LOD 0.5 ng ALP/ml plazme. Z LC-MS/MS tehniko smo dokazali izredno občutljivost za ALP (0.1 ng/ml plazme). LC-MS/MS je zato prav gotovo metoda prvega izbora za določanje ALP v plazmi pri farmakokinetičnih študijah.

Development of Chromatographic Methods Coupled with Mass Spectrometry for Determination of Alprazolam in Plasma

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These research represents development of analytical method for determination of alprazolam (ALP) in plasma. Alprazolam is antidepressant and anxiolitic drug. The aim of work was to develop sensitive, reproducible and linear method for quantitative determination of alprazolam in plazma for farmacokinetics studies. I used GC/MS analyse technique, which has a good resolution and sensitivity and additional reliable identification of molecule, that is mass specter. Developed GC/MS method were transferred on LC-MS/MS with aim to obtain higher sensitivity (lower LOQ) and specificy. Development of analytical method also includes preparation of plasma samples with SPE and L-L extraction. They were bouth sucessull. For L-L extraction butilchloride was used for organic solvent at pH 9. SPE extraction was performed on Strata-X extraction columns with optimal elution solvent acetonitrile. GC/MS method is linear in concentration range from 1 to 100 ng/ml of alprazolam in plasma. LOQ is 1 ng/ml and LOD is 0.5 ng/ml. A better sensitivity for alprazolam (LOQ 0.1 ng/ml in palsma) was obtained with LC-MS/MS technique. LC-MS/MS is definitely method of first choice for determination of alprazolam in plasma in farmacokinetics studies.

Določanje odpornosti bakterij *Campylobacter coli* proti ciprofloksacinu z metodo MAMA – PCR

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V razvitih državah narašča odpornost bakterij rodu *Campylobacter* proti fluorokinolonom zaradi njihove obsežne uporabe pri zdravljenju ljudi in v moderni prireji živali, namenjenih za prehrano človeka. Najpogostejši vir odpornih kampilobakterjev predstavlja okužena perutnina. Za vrsto *C. coli* je značilno, da je bolj odporna kot vrsta *C. jejuni*. Odpornost kampilobakterjev proti fluorokinolonu ciprofloksacinu povzroča točkovna mutacija v podenoti A gena DNA giraze. Za odkrivanje te mutacije na kodonu 86 so razvili hitro metodo, imenovano MAMA – PCR (Mismatch Amplification Mutation Assay PCR). V nalogi smo prilagodili pogoje postopka MAMA – PCR za odkrivanje mutacije pri vrsti *C. coli*. Uporabili smo nov začetni oligonukleotid CampygyrCOLIwild in določili pogoje postopka za odkrivanje nemutirane oblike kodona 86 gena gyrA bakterij *C. coli*. Rezultati pomnoževanja obeh oblik kodona so se ujemali pri 94,0% izolatov, zato nam je nov postopek za odkrivanje izolatov brez mutacije služil kot negativna kontrola metode MAMA – PCR. Primerjava fenotipskega ugotavljanja občutljivosti za ciprofloksacin in MAMA – PCR je pokazala, da se je ujemalo 84,5% rezultatov. 15,5% rezultatov se ni ujemalo bodisi zaradi napačne interpretacije fenotipskih testov ali zaradi neujemanja obeh postopkov. Menimo, da lahko metoda MAMA – PCR predstavlja učinkovito alternativno molekularno metodo za ugotavljanje odpornosti bakterij vrste *C. coli* proti ciprofloksacinu. Pred tem pa je potrebna primerjava rezultatov med različnimi laboratoriji.

Detection of Ciprofloxacin Resistance in *Campylobacter coli* by MAMA-PCR

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There is an increase in fluoroquinolone resistance in *Campylobacter* spp. in developed countries, which is driven by the extensive use of antimicrobial agents in medical treatment of people and in modern breeding of animals intended for human consumption. Contaminated poultry is the most common source of resistant *Campylobacter*. *C. coli* is generally more resistant than *C. jejuni*. Resistance to fluoroquinolone ciprofloxacin in campylobacters is mediated by a point mutation in the gene encoding the subunit A of the DNA gyrase. A rapid PCR method called MAMA – PCR (Mismatch Amplification Mutation Assay PCR) was developed for the detection of this mutation. In our study we modified the conditions of MAMA – PCR protocol for detection of the mutation in C. coli. We used a new primer CampygyrCOLIwild and determined the conditions of the protocol for the detection of isolates without the mutation at codon 86 in the gyrA gene of *C. coli*. The results of the amplification of the mutated and wild-type codon 86 matched in 94,0% of isolates. The new protocol for identification of strains without mutation served as a negative control of MAMA – PCR. The comparison of classical and MAMA – PCR susceptibility testing has shown the same result in 84,5% of cases. 15,5% of the results did not match either because of the incorrect interpretation of the results obtained by the classical technique, or because of the diverse results of both MAMA – PCR protocols. We belive that the MAMA – PCR can represent an efficient alternative molecular method for determining the resistance of C. coli bacteria to ciprofloxacin. Prior to that, a comparison of results of different laboratories would be necessary.

Delazmožnost po akutnem koronarnem sindromu in miokardni revaskularizaciji

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Z raziskavo smo ugotavljali, kakšen je vpliv telesnih, socialno-demografskih, psiholoških dejavnikov ter invazivne interventne obravnave akutnega koronarnega sindroma (AKS) na delazmožnost bolnikov po AKS.
V retrospektivno kohortno raziskavo smo zajeli 174 bolnikov z AKS, mlajših od 60 let in pred AKS zaposlenih za poln delovni čas ter vključenih v ambulantni rehabilitacijski program. Demografske podatke, podatke o zdravljenju in telesni zmogljivosti smo povzeli iz medicinske dokumentacije. Podatke o dejavnikih vračanja na delo smo pridobili z vprašalnikom. Popolno izpolnjen vprašalnik je vrnilo 82 bolnikov, od katerih se je na delo vrnilo 58 bolnikov. Tisti, ki so se vrnili na delo, so imeli redkeje sladkorno bolezen (9% vs. 29% p=0,02), pogosteje so bili zdravljeni z acetilsalicilno kislino (79% vs. 50%, p=0,01), imeli so večjo telesno zmogljivost pri obremenitvenem testiranju ob koncu rehabilitacije (8,6±2,4 MET vs. 7,4±1,9 MET, p=0,03), navajali so večjo zmogljivost za telesne obremenitve v vsakdanjem življenju (nošenje vreč 95% vs. 75%, p=0,01; težke dejavnosti 60% vs. 25%, p=0,004), bili so pogosteje deležni opore prijateljev (88% vs. 67%, p=0,02) in sodelavcev (67% vs. 38%, p=0,01), pogosteje so imeli veliko željo po vrnitvi na delo (50% vs. 25%, p=0,04) in so pogosteje prejeli zdravniški nasvet o vrnitvi na delo (72% vs. 8%, p<0,001). V multivariantni analizi sta vrnitev na delo napovedovala nasvet zdravnika (RO 63,9, 95% IZ 5,4-754, p=0,001) in velika želja po vrnitvi na delo (RO 7,3, 95% IZ 1,1-49,5, p=0,04). Način zdravljenja AKS na vračanje bolnikov na delo ni imel vpliva.

Return to Work after Acute Coronary Syndrome and Myocardial Revascularisation

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We evaluated how working ability is affected by early revascularisation therapy of acute coronary syndrome (ACS) and by several between physical, sociodemographic and psychical factors.
In retrospective cohort study we screened 174 patients. The data on clinical characteristics, the mode of treatment of ACS and exercise capacity were obtained from medical charts. The psychosocial factors and the data about working ability were gathered by structured questionnaire. Eighty-two patients returned complete questionnaire and 58 of them returned to work. The patients who returned to work had less diabetes (9% vs. 29%, p=0,02), were more frequently treated with acetylsalicylic acid in acute phase (79% vs. 50%, p=0,01), had higher exercise capacity after the rehabilitation (8,6±2,4 MET vs. 7,4±1,9 MET, p=0,03), and in everyday life (carrying bags 95% vs. 75%, p=0,01; heavy activities 60% vs. 25%, p=0,004), were more frequently supported by their friends (88% vs. 67%, p=0,02) and co-workers (67% vs. 38%, p=0,01), had greater desire to return to work (50% vs. 25%, p=0,04) and were more often advised to return to work by the doctor (72% vs. 8%, p<0,001). In multivariate analysis return to work was positively associated with doctors advice (OR 63,9, 95% CI 5,4-754, p=0,001) and greater desire to return to work (OR 7,3, 95% CI 1,1-49,5, p=0,04). There was no difference in working ability among patients who were treated invasively or conservatively in acute phase.

Vlaženje, ogrevanje in hlajenje procesnega zraka med fermentacijo

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Delo predstavlja rezultate meritev in izračunov parametrov za določitev masne bilance vlaženja zraka v fermentorjih.
Meritve smo izvajali v reaktorjih različnih volumnov od 23 L v laboratoriju, 150 L v pilotnem merilu in do 8 m^3 v industrijskem merilu. Poskusi v fermentorjih so potekali pri različnih obratovalnimi pogojih (intenzivnost mešanja in zračenja), z različnimi mediji ter pri različnih temperaturah.
Osnovne podatke o vlaženju zraka smo pridobili v laboratorijskem fermentorju, kakor tudi meritve za toplotni in snovni prenos. Iz meritev vlaženja zraka pri prehodu skozi fermentor smo ugotovili, da se v večini obratovalnih pogojev zrak nasičeno navlaži pri temperaturi medija. Osnovni namen poskusov je vzdrževanje konstantnega nivoja medija v fermentorjih med celotnim procesom. Računske vrednosti izračunane s pomočjo masne bilance smo primerjali z eksperimentalnimi vrednostmi in ugotovili ujemanja pri laboratorijskem fermentorju. Pri pilotnem in industrijskem fermentroju smo določili razliko odstopanja med vrednostmi in s pomočjo le tega uvedli korigirni faktor.
Za določitev volumskega koeficienta toplotne in snovne prestopnosti smo določili enačbe, s katerimi smo izračunali vrednosti za posamezna merila fermentorjev. S pomočjo določenih vrednosti ha in $k_c a$ v laboratorijskem fermentorju smo določili korelacijski enačbi, na osnovi katerih lahko sklepamo na majhen vpliv intenzivnosti mešanja na ha in $k_c a$.

Humidity and Heat Transfer During the Aeration of Fermentation Process

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The aim of measurements and calculation procedure was to define a mass balance of air humidification during the fermentation process.
The measurements were performed at three different scales, i.e. 23 L laboratory scale, 150 L pilot and 8 m^3 industrial scale fermenter, where influence of operating conditions such as mixing and air flow were studied with different media and at different temperatures regimes.
Initial humidity measurements and the heat and mass transfer results were obtained using laboratory scale fermenter. According to the results, we claim that air passing through the bioreactor during the fermentation process is saturated at the media temperature for most measured operating conditions.
When scaled-up to pilot and industrial fermenters differences between predicted and measured value occurred. The calculation procedure of a correction factor was proposed.
The calculation for heat and mass transfer coefficients are also reviewed. When evaluating value of ha and $k_c a$ in laboratory fermenter, we made correlations, which showed small influence of mixing on ha and $k_c a$.

Študij novih kristalnih oblik K-0903

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Iz predhodnih študij je znano, da zdravilna učinkovina K-0903 izkazuje polimorfizem. Znana sta dva polimorfa (metastabilna B in stabilna A oblika), dva solvata in amorfna oblika. Preiskovana učinkovina K-0903 je optično aktivna.
Namen naloge je priprava novih polimorfnih oblik racemata učinkovine K-0903 ter priprava in študij kristalizacije optično čistih enantiomerov.
Za karakterizacijo kristalnih oblik smo uporabili termično analizo (TGA in DSC), IR in ramansko spektroskopijo, rentgensko praškovno difrakcijo, hitrost raztapljanja, intrinzično hitrost raztapljanja (IDR) ter stični kot, izbrane kristalne oblike pa smo analizirali tudi s tekočinsko kromatografijo visoke ločljivosti (HPLC) in jim določili pravo gostoto ter dinamično sorpcijo vode.
S prekristalizacijo K-0903 iz topil i-propanol, i-propanol/voda in kloroform smo izolirali tri nove pseudopolimorfne modifikacije (solvate). Nekatere njihove lastnosti, kot npr. hitrost raztapljanja, so ugodnejše kot pri komercialno uporabljenem polimorfu A, vendar so nestabilne in ob fizikalnem ali kemičnem stresu prehajajo v stabilno obliko A.
Z asimetrično sintezo smo pridobili R- in S enantiomera. Kristalizirala sta v obliki seskvihidrata. To je termodinamsko najstabilnejša homokiralna kristalna oblika. Pri postopku sušenja z razprševanjem sta nastali amorfni obliki enantiomerov. IDR optično čistih enantiomerov, predvsem amorfnih vzorcev, je bistveno večja kot pri racematnih kristalnih in amorfni strukturi.

Study of New Crystal Structures of K-0903

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The preliminary studies have shown that the compound K-0903 exhibits polymorphism. Two polymorphs (metastable B and stable A), two solvates and amorphous form were known. The examined compound K-0903 is an optically active drug.
The aim of this study was to prepare new polymorphic forms of racemic compound K-0903, and to prepare and study the crystallization behaviour of optically pure enantiomers.
The obtained crystal structures were characterized by thermal analysis (TGA and DSC), IR and Raman spectroscopy, X-ray powder diffractometry, dissolution rate, intrinsic dissolution rate (IDR), contact angle, while some of the samples were also studied by high performance liquid chromatography (HPLC), true density and dynamic water sorption.
Three new pseudopolymorphic modifications (solvates) of compound K-0903 were obtained by recrystalization from different solvents: i-propanol, i-propanol/water and chloroform. Some of their properties, such as the dissolution rate, are more favourable then those by commercially used polymorph A, but they are unstable and when exposed to any kind of physical or chemical stress they convert into a stable form A.
R- and S- enantiomers were produced by asymmetric synthesis. They crystallize as sesquihydrates. Both are thermodinamically the most stable homochiral crystal structures. By spray drying we obtained the amorphous forms of single enantiomers. IDR is significantly higher in optically pure samples, especially in amorphous forms, compared to racemic crystal and amorphous forms.

Sinteza in načrtovanje novih fosfonatnih inhibitorjev mikoliltransferazne aktivnosti antigena 85C s potencialnim protituberkuloznim delovanjem

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V okviru raziskovalnega dela smo sintetizirali nove inhibitorje mikoliltransferazne aktivnosti antigena 85C s potencialnim protituberkuloznim delovanjem. Za spojino vodnico smo uporabili spojino etil 3-(fenoksi)benzil butilfosfonat, ki je bila predhodno sintetizirana kot mimetik prehodnega stanja naravnega substrata trehaloze monomikolata, in za katero se je izkazalo, da je njena *in vitro* protituberkulozna učinkovitost zelo dobra. Spojine smo načrtovali s pomočjo poznavanja strukture aktivnega mesta antigena 85C, kjer je prisotno vezavno mesto za trehalozo in za eno od alkilnih verig naravnega substrata (trehaloza monomikolat). Postavili smo hipotezo, da prisotnost skupin z visoko elektronsko gostoto na fenoksifenilnem skeletu bolje posnema naravni substrat in zato vodi do učinkovitejših inhibitorjev mikoliltransferazne aktivnosti antigena 85C v primerjavi s spojino vodnico. Pripravili smo metoksi derivate etil 3-(fenoksi)benzil butilfosfonata, za katere smo z molekulskim modeliranjem simulirali vezavo v aktivno mesto encima. *In vitro* meritve testov protituberkulozne učinkovitosti spojin bodo izvedene v laboratoriju Univerze v Birminghamu.

Synthesis and Developement of New Phosphonate Inhibitors of Mycolyltransferase Activity of the Antigene 85C with a Potential Antituberculous Activity

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In the scope of our research work new inhibitors of mycolyltransferase activity of the antigen 85C with a potential antituberculous activity were synthesized. As a lead compound ethyl 3-(phenoxy)benzyl butylphosphonate was used, which was synthesized previously as a transitional state mimetic of the natural substrate trehalose monomycolate and showed good antituberculous activity. The inhibitors were designed according to our knowledge of the structure of the antigen 85C which contains a binding site for trehalose and one of the alkyl branches of trehalose monomycolate. We postulated that placing functional groups with high electronic density on the phenoxyphenyl moiety would lead to better mimetics of the natural substrate and subsequently to more potent inhibitors of mycolyltransferase activity of antigen 85C in comparison with the lead compound. Several methoxy derivatives of the lead compound were prepared and molecular modelling was used to simulate their binding in the active site. *In vitro* antigen 85C inhibitory potency of the synthesized compounds will be tested in due course at The School of Biosciences, The University of Birmingham, UK.

Vpliv slanosti na transkripcijo genov za encime, ki sodelujejo pri modifikaciji maščobnih kislin pri halofilni črni kvasovki *Hortaea werneckii*

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Halofilna črna kvasovka *Hortaea werneckii*, v medicini znana kot povzročiteljica *tineae nigrae*, neinvazivne kožne infekcije, spada med askomicetne glive reda *Dothideales*. Njena naravna ekološka niša so izjemno slane vode morskih solin, kjer predstavlja dominantno glivno vrsto. Sposobna je rasti v nasičeni raztopini soli kot tudi v raztopini brez soli in zato predstavlja zelo primeren modelni evkariontski halofilni organizem. Povišana koncentracija soli v okolju v celicah te glive poleg ostalih sprememb sproži kopičenje glicerola kot kompatibilnega topljenca ter spremembe lastnosti membran. Predvsem se spremeni sestava maščobnih kislin, ki v povprečju pri višjih slanostih postanejo daljše in bolj nenasičene (zlasti na mestih Δ-9 in Δ-12). Preverjali smo hipotezo, da so spremembe v sestavi maščobnih kislin posledica spremenjenega izražanja genov, ki kodirajo encime, odgovorne za podaljšanje in nenasičenje maščobnih kislin. V genomu *H. werneckii* smo poiskali delna nukleotidna zaporedja za elongazo (*HwELO1*), Δ-9 desaturazo (*HwOLE1*) in Δ-12 desaturazo (*HwODE12*). Z analizo prenosa po Southernu smo ugotovili, da sta v genomu *H. werneckii* vsaj po dve kopiji vsakega od naštetih genov. Z verižno polimerazno reakcijo na cDNA prepisu celične mRNA smo preučili transkripcijo teh genov v celicah, vzgojenih pri različnih koncentracijah soli oziroma spremembe transkripcije v celicah po izpostavitvi osmotskemu šoku. Raziskava je pokazala, da maščobnokislinsko sestavo membran celic *H. werneckii* vsaj deloma uravnava transkripcijska kontrola izražanja genov, na katerih je zapis za tri pomembne encime, udeležene v metabolizmu maščobnih kislin.

The Influence of Salinity on the Transcription of Genes Encoding the Enzymes, Involved in Modifications of Fatty Acids in Halophilic Black Yeast *Hortaea Werneckii*

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Halophilic black yeast-like fungus *Hortaea werneckii*, also known as the causative agent of *tinea nigra*, a non-pathogenic superficial skin infection, occurs naturally in hypersaline waters of solar salterns where it represents the dominant fungal species. This halophilic species can grow at a wide range of salinities from zero to the point of saturation and is thus a highly appropriate model organism for the study of salt tolerance in eukaryotes. In this species, besides the accumulation of glycerol as a compatible solute, different responses to increased salinity were observed, among others also at the level of membrane composition and fluidity. An increase in fatty acid unsaturation and length were observed at greater salinities. These modifications were assumed to be the consequence of alterations in fatty-acid-modifying enzymes' expression. In present study, partial DNA sequences of the homologue genes for fatty acid elongase (*HwELO1*), Δ-9 and Δ-12 desaturases (*HwOLE1* and *HwODE12*, respectively) were found. It was demonstrated by Southern blot analysis of the genomic DNA, that there are at least two copies of the *HwELO1*, *HwOLE1* and *HwODE12* genes in the genome of *H. werneckii*. Differences in transcription of the examined genes in cells grown at different salinities and changes in transcription following osmotic shock were investigated by reverse transcription polymerase chain reaction (RT-PCR). Our research showed that the fatty acid composition of the *H. werneckii* cells is at least partially regulated through the transcription control of genes, encoding three important fatty-acid-modifying enzymes.

Sinteza in karakterizacija 2-aminotiazolnih in aminokinonskih derivatov – potencialnih biološko aktivnih spojin

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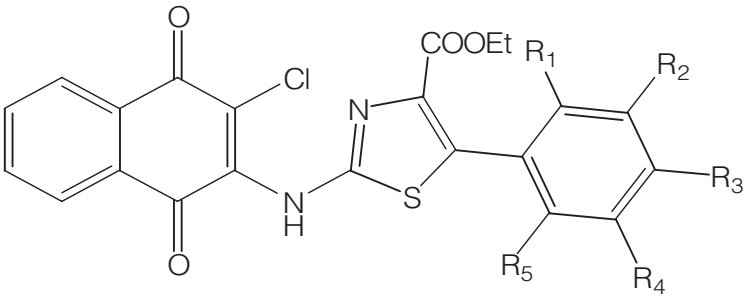
Pri našem delu smo izvedli sintezno pot od enostavnih benzaldehidov do aminokinonskih derivatov in analizirali vse sintetizirne spojine, in sicer z določitvijo temperature tališča, IR in ^{1}H NMR spektroskopijo, nekatere pa tudi z UV/VIS spektroskopijo, masno spekrometrijo in elementno analizo.
Iz nekaterih komercialno dosegljivih benzaldehidov smo v začetni fazi s Knoevenaglovo kondenzacijo sintetizirali etilenske spojine. V nadaljevanju smo s pomočjo natrijevega klorata(I) iz etilenskih spojin pripravili oksirane. Na te smo učinkovali s tiosečnino kot binukleofilom, pri čemer so nastali ustrezni derivati 2-aminotiazolov. Naš končen produkt so aminokinonski derivati, ki smo jih pridobili iz 2,3-dikloro-1,4-naftokinona in predhodno pripravljenih derivatov 2-aminotiazolov (shema 1).
Tako derivati 2-aminotiazolov, kot tudi aminokinonski derivati, so spojine z različnimi biološkimi učinki in se uporabljajo kot zdravilne učinkovine pri številnih obolenjih.

R_1	R_2	R_3	R_4	R_5
H	OMe	H	H	OMe
H	H	Cl	H	H
H	H	NO_2	H	H
H	H	CH_3	H	H
H	H	H	H	H



Shema 1: Sintetizirani aminokinonski derivati

Synthesis and Characterization of 2-aminothiazole and Aminoquinonic Derivatives – Potential Biological Active Compounds

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We have performed a synthetic approach from the simple benzaldehydes towards aminoquinonic derivatives. The structures of all isolated compounds was confirmed by various analytical methods including IR-spectroscopy, ^{1}H NMR-spectroscopy and melting point determination, for some of the products also mass spectrometry, UV/VIS spectroscopy and elemental analysis were performed.
In the initial stage we have synthesized the ethylenic compounds starting from commercially available benzaldehydes using the Knoevenagel condensation procedure. In the following step the ethylenic compounds were treated with an aqueous solution of sodium chlorate(I) yielding oxirane derivatives.

The reactions between oxiranes and thiourea as nucleophilic reagents resulted in formation of 2-aminothiazoles. The aminoquinonic derivatives, the goal products of our synthesis, were obtained by treatment of 2-aminothiazoles with the 2,3-dichloro-1,4-naphtoquinone (scheme 1).
2-Aminothiazoles as well as aminoqiunonic derivatives present groups of compounds with various biological effects and are already used as drugs in numerous illness.

Vpliv klodronata in EDTA na paracelularni transport aciklovirja in fluoresceina skozi steno jejunuma podgane *in vitro*

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Klodronat in EDTA sta spojini, ki sta sposobni vezave dvovalentnih ionov. Ti ioni so prisotni v strukturah medceličnih povezav med epitelijskimi celicami in odvzem le-teh povzroči odprtje tesnih stikov. V raziskovalni nalogi smo proučevali, kako ti dve spojini vplivata na odprtje tesnih stikov. Odprtost tesnih stikov smo ocenjevali s povečanjem prehoda paracelularnih označevalcev, v našem primeru sta bila to aciklovir in fluorescein.
Ugotovili smo, da EDTA statistično signifikantno poveča prehod paracelularnih označevalcev aciklovirja in fluoresceina pri koncentraciji 3 mM oziroma 2 mM. Nadaljnje povečevanje koncentracije EDTA povečuje paracelularni prehod označevalcev, a le do koncentracije 10 mM, kjer dosežemo maksimalen učinek. Tega višje koncentracije več ne spremenijo. EDTA je tudi toksičen za jejunum podgane, saj 80-minutni tretma z 2 mM koncentracijo preživi le 50% tkiv. Pri 3 mM koncentraciji pa vitalnih tkiv ni več.
Klodronat ima podobne učinke na paracelularni prehod kot EDTA. Statistično signifikantno povečani prehod aciklovirja in fluoresceina zabeležimo pri koncentraciji 10 mM oziroma 15 mM. Maksimalni učinek dosežemo pri 25 mM koncentraciji. Že pri 20 mM koncentraciji lahko opazimo toksične učinke na jejunum podgane – število vitalnih tkiv se zmanjša na 75%. 30 mM koncentracija pa je toksična za vsa tkiva. Toksičnost klodronata je posledica vezave dvovalentnih ionov in ne absorpcije v enterocite, kjer se metabolizira v citotoksični metabolit.

Effect of Clodronate and EDTA on Paracellular Transport of Acyclovir and Fluoresceine Through the Rat Jejunum *In vitro*

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Clodronate and EDTA are compounds capable of binding metallic cations. Calcium is a structural part of a junctional complex among the epithelial cells and its depletion results in the opening of the tight junctions. In this research we have studied the influence of these two compounds on the tight junctions by monitoring the transport of paracellular markers (acyclovir and fluorescein) through the rat jejunum *in vitro*.
EDTA significantly increases the transport of paracellular markers through rat jejunum at concentration levels of 3 mM for acyclovir and 2 mM for fluorescein. Higher concentrations further increase the permeability of markers until a plateau is reached at 10 mM concentration. EDTA is also toxic for the rat jejunum. Namely, in 80-minute treatment at 2 mM concentration only 50% of isolated tissue segments remain viable. At 3 mM concentration, no viability was detected.
Clodronate has similar effect on the paracellular permeability as EDTA. Significantly increased transport of paracellular markers through the epithelium was detected at 15 mM concentration for acyclovir and 10 mM concentration for fluorescein, a plateau was reached at 25 mM concentration. A toxic effect was detected at 20 mM clodronate, when the viability of 1 out of 4 tested tissue segments was impaired. When the concentration was increased to 30 mM, no viable tissue was detected. The toxicity is most likely a consequence of the depletion of bivalent cations and not of the absorption of clodronate to enterocytes, where it is metabolized into cytotoxic ATP analogue.

Optimizacija določevanja aminokislinske sestave s tekočinsko kromatografijo

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Ena izmed možnosti odstranitve površinskih nečistoč na svili in volni je obdelava z laserjem. Z lasersko obdelavo namreč lahko dosežemo brezkontaktno čiščenje materialov, ker pa pri tem lahko pride do kemijskih in ne le strukturnih poškodb materiala, sem želel preveriti, če so vidne razlike v aminokislinski sestavi. Zanimala me je tudi razlika v aminokislinski sestavi med ovčjimi in kravjimi siri ter razlike v aminokislinski sestavi različnih vrst želatin. V ta namen sem optimiziral pogoje hidrolize, pogoje derivatizacije nastalih prostih aminokislin z reagentom o-ftaldialdehidom/merkaptopropanojsko kislino (OPA-MPA) in separacijo nastalih derivatov z visokotlačno tekočinsko kromatografijo. Vzorce proteina znane sestave (govejega serumskega albumina), svile, volne, sira in želatine sem hidroliziral v koncentrirani HCl, ki sem jo segreval na 80 °C in dosegel popoln razcep peptidnih vezi v 24 h. Opazil sem, da po tem postopku hidrolize ni mogoče določevati asparagina in glutamina, ker se pretvorita v asparaginsko in glutaminsko kislino, ter triptofana in cisteina, ker triptofan med kislinsko hidrolizo razpade, cistein pa se v prisotnosti zračnega kisika oksidira. Oksidacijo cisteina med hidrolizo sem zmanjšal z uporabo inertne atmosfere.
Proste aminokisline sem derivatiziral z OPA-MPA reagentom v boratnem pufru pri pH 9. Prolina in hidroksiprolina z OPA-MPA reagentom ni mogoče derivatizirati, ker sta sekundarni aminokislini.
Ugotovil sem, da obsevanje svile in volne z laserjem ne spremeni aminokislinske sestave tistih aminokislin, ki sem jih lahko določeval. Med siri kravjega oz. ovčjega porekla v aminokislinski sestavi nisem opazil razlike. Želatine se med seboj najbolj razlikujejo po vsebnosti Glu+Gln. Bistvenih razlik v aminokislinski sestavi želatin nisem opazil.

Determination of Amino Acid Composition with Liquid Chromatography

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One of the possibility for cleaning silk or wool is irradiation with laser. Alterations in amino acid composition which could arise in the process of irradiation were investigated.
The work was focused on optimisation of acidic hydrolysis of proteins in biological samples, on conditions for derivatisation with o-phthaldialdehyde/3-mercaptopropionic acid reagent (OPA-MPA) and on the chromatographic separation of derivates. Samples of a protein with a well-known composition (bovine serum albumine), silk, wool, cheese and gelatine were hydrolysed in concentrated HCl at 80 °C for 24 h. It turned out that acidic hydrolysis induces transformation of asparagine and glutamine to aspartic and glutamic acid respectively, decomposition of tryptophan and oxidation of cystein. Oxidation of cystein can be minimised using inert atmosphere. Amino acids were derivatised with OPA-MPA reagent in borate buffer solution with pH 9. Proline and hydroxyproline, being secondary amino acids, cannot be derivatised with the reagent because of their specific structural properties. The method was further used for determination of amino acid composition of silk, wool, cheese and gelatine. There was no significant alteration found in amino acid composition of irradiated or non-irradiated silk and wool samples.

Dokazovanje pestivirusov z metodo verižne reakcije s polimerazo v realnem času

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Pestivirusi so majhni virusi z enoverižno RNA, ki je velika okoli 12,5 Kb. Genom ima en odprt bralni okvir z zelo dobro ohranjeno 5´- in 3´- nekodirajočo regijo. V rod *Pestivirus* (družina *Flaviviridae*) spadajo virus klasične prašičje kuge, virus bovine virusne diareje in virus borderske bolezni ovac. Klasična prašičja kuga je zelo nalezljiva bolezen domačih prašičev in povzroča velike ekonomske izgube v prašičereji po celem svetu. Za ugotavljanje izbruhov klasične prašičje kuge in za ločevanje virusa KPK od ostalih pestivirusov potrebujemo hitre, občutljive in specifične laboratorijske metode.
Metoda PCR v realnem času je natančna, hitra in zanesljiva, uporabimo pa jo lahko za kvantifikacijo točno določenih molekul DNA. Metoda temelji na sprotnem merjenju fluorescenčnega signala, ki je sorazmeren s količino pomnoženega produkta. Nastajanje produktov PCR lahko spremljamo z nespecifičnim barvilom SYBR® Green in s specifičnimi sondami TaqMan®. Pri našem delu smo optimizirali obe metodi. Z obema smo dokazali prisotnost vseh pestivirusov izoliranih iz celičnih kultur ter ločili med virusom KPK in ostalimi pestivirusi. Pri detekcijski metodi s sondami TaqMan® smo metodo optimizirali tako, da smo rezultate lahko uporabili za kvantifikacijo virusne nukleinske kisline.

Development of Real-Time PCR for Detection of Pestiviruses

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Pestiviruses are small enveloped viruses containing positive sense single stranded RNA of approximately 12.5 Kb in length. Their genomes have a large open reading frame flanked by highly conserved 5' and 3' non-coding regions. The *Pestivirus* genus (family *Flaviviridae*) comprises three species, namely classical swine fever virus, bovine viral diarrhoea virus and border disease virus. Classical swine fever is a highly contagious disease of domestic pigs that has been responsible for large economic losses in different parts of the world. Rapid, sensitive and specific laboratory diagnostic methods are necessary to confirm outbreaks of CSF and to distinguish mild forms of the disease from infections caused by other pestiviruses.
Real-time PCR is an accurate, rapid and reliable method that can be used for the quantification of specific DNA molecules. The basic principle is the recurring measurement of a fluorescent signal, which is proportional to the amount of amplification product. Nonspecific ds-DNA-binding dye SYBR® Green or specific TaqMan® probes can be used. We optimised both of the detection methods. With SYBR® Green and TaqMan® probes we detected all of the pestiviruses isolated from cell cultures and we could also distinguish CSFV from other pestiviruses. The TaqMan® method was optimised and used for the quantification of the PCR products.

Možnosti kompostiranja blata iz industrijske čistilne naprave

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Namen raziskovalnega dela je preučiti možnosti kompostiranja blata, ki nastane kot stranski produkt pri čiščenju odpadnih voda. Kompostno mešanico smo pripravili iz blata in pomožnega materiala, v našem primeru drevesnega lubja, ki smo ju zmešali v prostorninskem razmerju 1 : 1,2. Dobro homogenizirano kompostno mešanico smo dodali v pilotni reaktor s prostornino 1,3 m^3, v katerega smo neprekinjeno uvajali zrak. Kompostirali smo 23 dni. Med kompostiranjem smo spremljali izgubo mase, temperaturo in pH vrednost. Ob zaključku procesa smo kompostno mešanico presejali in del pomožnega materiala uporabili v naslednjem poskusu. Po istem postopku smo izvedli tri poskuse kompostiranja in pri vsakem opravili meritve na vzorcu začetne kompostne mešanice, na vzorcih komposta in na izlužkih, ki smo jih pripravili iz teh vzorcev.
Iz dobljenih rezultatov lahko ugotovimo, da je kompostiranje primeren način obdelave odpadnega blata, saj se v treh tednih razgradi do 71,9% organskih snovi. Glede na slovensko zakonodajo lahko dobljeni kompost uvrstimo v skupino nenevarnih odpadkov. Kompost s takimi lastnostmi je primeren za odlaganje na kmetijskih površinah.

The Composting Possibilities of the Sludge from Industrial Waste Water Treatment Plant

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The purpose of the research work was to investigate the possibility of composting the sludge, which remained as a side product of waste water treatment technology. The compost mixture was prepared from sludge and from the subsidiary material, in our case tree bark, at volume ratio 1 : 1,2. Well homogeneus compost mixture was added to a pilot reactor with volume 1,3 m^3 which was continually aerated.
The composting process during 23 days was observed over a loss of the mass, temperature and pH measurements. Compost mixture was sifted at the end of the process and a part of subsidiary material was used for the following experiment. Three experiments were carried out using the same procedure and the measurements were performed on the samples from the starting compost mixture, the compost and leach samples.
The results show that organic compounds were reduced in three weeks by 71,9%. Therefore, the compost process is a suitable way of sludge treatment. According to Slovenian legislation the obtained compost is ranged among non-hazardous wastes and also is suitable for disposal on the agricultural areas.

Priprava in analiza DNA-mikromrež homeostaze holesterola

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V diplomski nalogi smo izdelali specifične sonde za STEROTALK cDNA-mikromrežo, na kateri so imobilizirani fragmenti cDNA genov homeostaze holesterola. S polimerazno verižno reakcijo s sondno-specifičnimi začetnimi oligonukleotidi smo pomnožili fragmente genov iz tkivno-specifičnih cDNA knjižnic, jih klonirali in jim preverili nukleotidno zaporedje. Tako smo ustvarili banko bakterijskih klonov, ki omogoča serijsko izdelavo cDNA- mikromrež z nanosom ustreznih sond na stekleni nosilec z robotom. Kvaliteto naših mikromrež smo preverili s hibridizacijo dveh različnih mišjih referenčnih vzorcev RNA. Obe RNA smo obratno prepisali v cDNA v prisotnosti fluorescentno označenih nukleotidov (cianin-3-dCTP ali cianin-5-dCTP) in ju ko-hibridizirali na mikromreže. S pomočjo laserja s konfokalno optiko smo optično odčitali intenzitete fluorescenc sond, ki so sorazmerne z relativno stopnjo izražanja posameznih genov v vzorcu. Eden od problemov pri analizi podatkov mikromrež je njihova velika variabilnost, ki izvira iz številnih sistemskih napak, ki se kopičijo med postopkom izdelave mikromrež, hibridizacije in optičnega odčitavanja. Te sistemske napake lahko v precejšni meri odpravimo s postopkom normalizacije. V našem primeru smo podatke normalizirali na zunanje kontrole in pokazali, da uporaba kvalitetnih zunanjih kontrol omogoča večjo primerljivost med hibridizacijami.

Preparing and Analysis of Cholesterol Homeostasis DNA-Microarrays

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For the purpose of this thesis, specific probes were produced for the STEROTALK cDNA microarray containing immobilised fragments of cDNA genes, which are responsible for cholesterol homeostasis. Using polymerase chain reaction with probe specific primer oligonucleotides, we amplified the gene fragments from tissue-specific cDNA libraries, cloned them and checked the nucleotide sequence. Using this process, we establish a bank of bacterial clones, enabling us to produce cDNA microarrays in series by applying suitable probes onto the glass carrier with a robot. The quality of our microarrays was checked using hybridisation of two different reference RNA samples from mice. Both RNAs were reversely transcribed into cDNA in the presence of nucleotides with fluorescent markers (cyanine-3-dCTP or cyanine-5-dCTP) and co-hybridised them on the microarrays. Using confocal laser, spot intensities were read optically. The light intensity is directly related to the relative expression level of individual genes in the sample. A specific problem when analysing microarray data is its great variability, arising from numerous systemic errors, which increase with each process of microarray production, hybridisation and optical reading. These systemic errors can be reduced to a significant extent using normalisation. In our case, the data was normalised by using external controls, thus demonstrating that using high-quality external controls makes a higher degree of comparability between hybridisations possible.

Načrtovanje, sinteza in vrednotenje inhibitorjev rekombinantne 17ß-hidroksisteroid dehidrogenaze iz glive *Cochliobolus lunatus*

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17β-hidroksisteroid-dehidrogenaza iz glive *Cochliobolus lunatus* je regiospecifičen encim iz naddružine kratkoverižnih dehidrogenaz-reduktaz. Zaradi podobnosti v aminokislinskem zaporedju z nekaterimi človeškimi 17β-hidroksisteroid-dehidrogenazami, ki sodelujejo pri nastanku hormonsko odvisnih vrst raka, se ta glivni encim uporablja za preizkušanje potencialnih inhibitorjev človeških encimov. Zaradi strukturne podobnosti flavonoidov, znanih inhibitorjev 17β-hidroksisteroid-dehidrogenaz, z estri cimetne kisline, smo sintetizirali nekaj spojin iz te skupine in jih preizkusiti kot inhibitorje glivne 17β-hidroksisteroid-dehidrogenaze. Kinetična študija najbolj aktivne sintetizirane spojine je pokazala, da inhibira v smeri oksidacije, da je mesto vezave v encimu enako vezavnemu mestu substrata in da ni substrat za encim. S programoma Autodock 3.0 in CHARMm smo napovedali položaj in 3D orientacijo aktivnih spojin v encimu in poskušali razložiti razlike v steričnih lastnostih inhibitorjev in neinhibitorjev. Med položaji, ki jih je predlagal program Autodock, je pri najboljših inhibitorjih izstopal »zvit« položaj. Ta položaj zahteva fleksibilnost v predelu ob estrski vezi, zaradi katere se karbonilni kisik estrske vezi v encimu orientira tako, da omogoči nastanek vodikovih vezi med estrom in hidroksi skupinami Ser 153, Tyr 167 in Tyr 212. Za manj fleksibilne aktivne spojine pride v poštev »iztegnjeni« položaj, ki je zelo ugoden. Odvisnosti med aktivnostjo spojin in energijami vezave nismo odkrili. Razlike med aktivnimi in neaktivnimi spojinami smo našli v prisotnosti/ odsotnosti »zvitega« položaja in v večji prostorski razpršenosti prileganih neaktivnih spojinah. Kaže, da je računalniška analiza zelo koristen element pri načrtovanju učinkovitih inhibitorjev 17β-HSDcl, še posebej, če je povezana z dobrim poznavanjem katalitskega mehanizma.

Design, Synthesis and Evaluation of Inhibitors of Recombinant 17ß-Hydroxysteroid-Dehydrogenase from the Fungus *Cochliobolus lunatus*

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17β-hydroxysteroid-dehydrogenase from fungus *Cochliobolus lunatus* is a regiospecific enzyme from the shortchain dehydrogenase-reductase superfamily. Due to its similarity in aminoacid sequence with human 17β-hydroxysteroid-dehydrogenases, which participate in the formation of hormonally dependent forms of cancer, this fungal enzyme is used for testing potential inhibitors of human enzymes. Because of structural similarities of flavonoides, known inhibitors of 17β-hydroxysteroid-dehydrogenases, with esters of cynnamic acid, we synthesized some compounds from this group and tested them as inhibitors of fungal 17β-hydroxysteroid-dehydrogenase. Kinetic study of the most potent compund showed inhibition in the direction of oxidation. We also found that inhibitor most probably binds to the binding site of the substrate and is not substrate for the enzyme itself. With programs Autodock 3.0 and CHARMm we predicted position and 3D orientation of inhibitors in the enzyme and tried to explain differences in the sterical properties of inhibitors and non inhibitors. For the most potent inhibitors program Autodock suggested the »folded« conformation. This conformation needs some flexibility in the region near the ester bond. Because of this flexibility, the ester carbonyl oxygen can orientate in the enzyme in a way, which facilitates the formation of hydrogen bonds between the ester and hydroxy groups of Ser 153, Tyr 167 and Tyr 212. For more rigid potent structures the »extdended« conformation is better. We found no correlation between the activity of compounds and their docking energies. We found differences between active and inacitive compounds in presence/absence of the »folded« conformation and in greater spatial dispersity of docked inactive compounds. It seems that computer analysis is a useful element in the design of potent inhibitors of these enzymes.

Organizacija izobraževanja v podjetju Krka

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Izobraževanje in vseživljenjsko učenje zaposlenih je pomembno tako za razvoj in uspeh podjetja kot za razvoj zaposlenega, saj mu znanje omogoča uspešno in ustvarjalno delo.
Cilj raziskovalnega dela je bila kritična analiza organizacije izobraževanja zaposlenih v podjetju Krka, d. d. Ugotavljala sem potek in organiziranost izobraževanja, kdo so udeleženci, katere so najpogostejše oblike in metode izobraževanja, koliko sredstev za to namenijo, kakšni so učinki.
Z metodo intervjuja in iz zbranih sekundarnih podatkov oziroma virov sem ugotovila, da dajejo v Krki izobraževanju zaposlenih velik poudarek. Pri tem ima pomembno vlogo Izobraževalni center, ki skupaj s kadrovsko službo ter vodilnimi in vodstvenimi delavci načrtuje in organizira izobraževanje v skladu s finančnimi sredstvi, ki so za to namenjena.
Strokovno izobraženi in usposobljeni kadri so temeljni dejavnik razvoja, kakovosti in uspešnosti podjetja, zato je bistvenega pomena, da se zaposleni v podjetju nenehno izobražujejo, izpopolnjujejo in usposabljajo, saj bodo le tako zmožni opravljati svoje delo v skladu z zahtevami podjetja in sposobni slediti novim smernicam v prihodnosti.

The Organization of Education in Krka

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Education and continuous learning of the employees are just as important for the growth and the success of the company as for the greater proficiency of the employees because knowledge enables them to be sucessful and creative.
The focus of my research is the critical analysis of the organization of education in Krka. I have tried to establish the following: the organization of education in practice, who are the participants, which are the most common ways and methods of education, how many funds have been earmarked for the education of the employees, and how effective is the education of the employees.
By means of the question-answer method and the available secondary sources I was able to establish that Krka attributes a considerable importance to the education of its employees. The Education Centre, which with the help of the personnel department, the management, and the administration correctly plans and organizes education in concordance with the funds earmarked for this purpose, has an important part here.
The experts and the properly qualified present the basis for the growth, the quality, and the success of the company. Therefore, it is of utmost importance that the company organizes constant education, supports learnig, and qualification by education and experience. Only in this way the employees will be able to do their work according to the demands of the company and thus follow the new guidelines to the future.

Vpliv parfuma na razvoj in delovanje razmnoževalnih organov pri miškah

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Vomeronazalni organ je cevast organ, odgovoren za zaznavanje feromonov, ki so pomembni za komunikacijo med živalmi iste vrste. Feromoni delujejo na čutne celice v vomeronazalnem epiteliju, dražljaji se nato prenesejo v pomožni vohalni betič, od tu pa neposredno v hipotalamus in druge dele možganov, ki so povezani z delovanjem endokrinega oziroma spolnega sistema. V opisani raziskavi smo želeli preveriti, ali lahko stalna izpostavljenost močnemu vonju kot je parfum, vpliva na delovanje vomeronazalnega organa in posredno na razmnoževalno sposobnost in na razvoj spolnih organov pri miškah. Poskusno skupino so sestavljali pari mišk, ki so bile stalno izpostavljene parfumu v kletkah s filterskimi pokrovi. Kontrolne skupine so predstavljale miške, ki so bile gojene v enakih pogojih v ločenem prostoru brez parfuma. Mladiče moškega spola smo žrtvovali pri starosti 7, 20 in 50 dni, ter jim odvzeli moda za histološke in imunohistokemične preiskave, s katerimi smo ugotavljali izraženost 3β-hidroksisteroidne dehidrogenaze, antimülerjevega hormona in PCNA (proliferating cell nuclear antigen). Posebno skupino odraslih samcev smo za dvanajst dni izpostavili parfumu, ter jim po 4, 8 in 12 dneh odvzeli kri in izmerili raven testosterona. Izmerili smo premer semenskih cevk pri 50 dni starih samcih. Število mladičev in dolžina premora med gnezditvama se ni razlikovala med skupinama. Pri poskusnih živalih smo ugotovili nižje vrednosti testosterona kot pri živalih iz kontrolne skupine, vendar so vrednosti zelo nihale in razlike niso bile statistično značilne. Z imunohistokemičnimi metodami v modih potomcev parjenih živali nismo ugotovili razlik med poskusno in kontrolno skupino, ki bi kazale na motnje v dozorevanju mod po rojstvu, vendar pa smo ugotovili manjši premer semenskih cevk pri poskusni skupini samcev v primerjavi z modi samcev iz kontrolne skupine.

The Influence of Perfume on Development and Function of Reproductive Organs in Mice

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The vomeronasal organ is a small tubular organ, filled with mucosa, responsible for detecting pheromones. Pheromonal information sensed by vomeronasal organ is transmitted to the accessory olfactory bulb and from there to the other regions in the brain, including hypothalamus. The aim of our study was to establish whether permanent exposure to strong odour such as perfume, can influence reproductive capacity and development of reproductive organs in mice. Both control and experimental groups were composed of pairs of mice, caged together in cages with filter lids. Experimental groups were constantly exposed to perfume through filters. Control groups were bred in the same conditions in separate rooms. Male offspring were sacrificed on days 7, 20 and 50 post natally. Tests were used for immunocytochemical staining for 3β-HSD (3β-hidroxysteroid dehidrogenase), AMH (antimüllerian hormone) and PCNA (proliferating cell nuclear antigen). In the tests from 50 days old mice, seminiferous tubule diameter was measured. A separate group of male mice was under influence of perfume for 12 days and blood samples for testosterone measurements were taken after 4, 8 and 12 days. There was no difference observed either in litter size or time between litters between control and experimental groups. Levels of testosterone were lower in experimental group; however, due to high variability between individuals, the difference did not reach statistical significance. Immunocytochemical staining did not reveal any difference between experimental and control group. The only difference we found was in seminiferous tubule diameter, which was significantly smaller in the testes from experimental group of mice.

Ugotavljanje citotoksične aktivnosti nekaterih fitoestrogenov in derivatov cimetne kisline

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Novejše raziskave kažejo, da imajo številne naravne flavonoidne spojine potencialno protitumorno delovanje ter so zato dobro izhodišče pri iskanje spojin vodnic za razvoj kemoterapevtikov. 17β-hidroksisteroid dehidrogenaze so vpletene v zadnjo stopnjo sinteze spolnih hormonov. Imajo ključno vlogo pri uravnavanju hormonskega ravnovesja in so zato terapevtska tarča za nadzorovanje hormonsko pogojenih bolezni vključno z rakom dojke, endometrija in prostate.
V raziskavo potencialnega protitumornega delovanja smo vključili derivate cimetne kisline iz banke spojin Fakultete za farmacijo ter nekatere naravne flavonoide in fitoestrogene. Spojinam smo določili *in vitro* citotoksično delovanje na tumorske celične linije: MCF-7 (od estrogena odvisne celice adenokarcinoma dojke), MDA-MB-231 (od estrogena neodvisne celice adenokarcinoma dojke), Hep G2 (celice jetrnega hepatoma) in na celično linijo normalnih endotelnih celic (HUVEC). Eksponentno rastoče celice smo izpostavili spojinam (10^{-5} ali 10^{-6} M) in po 72 urah določili citotoksičnost s kolorimetrično metodo (MTT test). Dva derivata cimetne kisline GKK4 in GKK6 in spojini daidzein ter genistein iz skupine izoflavonoidov so selektivno zavrli rast od estrogena odvisnih celic MCF-7. Spojini 3-hidroksiflavon in derivat cimetne kisline GKK3, ki veljata za učinkovita inhibitorja 17β-hidroksisteroid dehidrogenaz, sta spodbudili celično proliferacijo od estrogena odvisnih celic MCF-7, kar kaže na možno rezidualno steroidogene aktivnosti omenjenih spojin.

Determination of Cytotoxic Activity of Some Phytoestrogens and Cinnamic Acid Derivatives

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Recent studies show that numerous natural flavonoid compounds have antitumor activity therefore representing a good base for searching for lead compounds for the development of chemotherapeutics. The 17β-hydroxysteroid dehydrogenases are involved in the last step of the biosynthesis of sex hormones. They have key role in hormonal regulation and represents emerging therapeutic target for the control of hormone dependent diseases including breast, endometrial and prostate cancer.
In the study for potential antitumor activity we included cinnamic acid derivatives from the bank of compounds of the Faculty of Pharmacy and several natural flavonoids and phytoestrogens. For several cinnamic acid derivatives it has been previously shown to be nonsteroidal inhibitors of 17β-hydroxysteroid dehydrogenases. The compounds were tested for their *in vitro* cytotoxic activity against cancer cell lines: MCF-7 (an oestrogen dependent breast adenocarcinoma cell line), MDA-MB-231 (an oestrogen independent breast adenocarcinoma cell line), Hep G2 (human hepatoma cell line) and a normal cell line HUVEC (human endothelial cells). The exponentially growing cells were exposed to the compounds (10^{-5} or 10^{-6} M) for 72 hours and cytotoxic activities were determined by a tetrazolium-based colorimetric assay (MTT assay). Cinnamic acid derivatives GKK4 and GKK6 and isoflavonoids genistein and daidzein selectively inhibited growth of oestrogen dependent MCF-7 cells. 3-hydroxyflavone and the cinnamic acid derivative GKK3, which have been shown to be inhibitors of 17β-hydroksisteroid dehydrogenases selectively stimulated proliferation of oestrogen dependent MCF-7 cells, which indicates residual steroidogenic activity of these compounds.

Tehnologija ločevanja organskih produktov s tankoslojnim uparevanjem

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2-(2-metoksifenoksi)etilamin, MFEA je reagent v sintezi aktivne farmacevtske učinkovine, ki se uporablja za zdravljenje hipertenzije. Kot izhodišče za čiščenje MFEA smo upoštevali, da je proces čiščenja omejen z dvema procesnima faktorjema, in sicer s temperaturo in z razpoložljivim vakuumom. Oba faktorja moramo upoštevati pri načrtovanju postopka čiščenja MFEA, tako da ne uporabimo previsoke delovne temperature, kar posledično pomeni uporabo nizkih tlakov. Študirali smo destilacijo na tankoslojnem uparjalniku. Najprej smo s šaržno laboratorijsko destilacijo določili obratovalne pogoje: tlak, temperaturo oljne kopeli, temperaturo hlapov destilata ter analizne metode za vrednotenje kvalitete produkta. Nato smo ugotovljene obratovalne pogoje ter analizne pristope uporabili pri destilaciji na tankoslojnem uparjalniku. Ugotovili smo, da sprejemljivo kvaliteto MFEA dobimo z naslednjimi obratovalnimi pogoji: temperatura termostata 135 °C, temperatura hlapov destilata 82 °C, tlak 30 mbar in hitrost dokapavanja zmesi 200 ml/h. Če bi razpolagali z boljšo vakuumsko opremo, bi lahko dosegli boljšo kvaliteto MFEA. S ponovno destilacijo destilatov in ostankov bi pridobili boljšo kvaliteto MFEA. Za napoved industrijske kapacitete smo kot povečevalni kriterij upoštevali dotok zmesi na enoto površine toplotnega menjalnika. Ob grelni površini industrijskega uparjalnika 1 m^2, temperaturi termostata 135 °C in tlaku 1-3mbar je za doseganje sprejemljive kvalitete, ki smo jo določili na min. 97 ut%, je dotok napajalne zmesi 12 l/h.

Technology of Organic Product Separation with Thin Film Evaporator

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2-(2-methoxyphenoxy)ethylamine, MFEA is the reagent in the synthesis of an active pharmaceutical ingredient, product, which is used for hypertension treatment. The quality of separated MFEA is limited by two factors, the available vacuum and applied temperature. Namely, at the temperatures higher than 140 °C degradation by products are detected. We studied the operating conditions: the pressure, the temperature of oil bath, the temperature of the distillate vapor and the methods of the analysis for the product quality evaluation. Obtained technological parameters were used for Thin Film Evaporation studies. The analysis showed that the optimal operating parameters are the following: the thermostat temperature 135 °C, the vapor temperature 82 °C, the pressure 30 mbar, dropping speed (ϕ) 200 ml/h and the area of Thin Film Evaporator 0,016 m^2. Generally, the highest level of product purity was produced, when lower pressure was applied. For better results we would need vacuum equipment. Product of better quality could be reached using an additional distillation of obtained product. On the basis of a pilot apparatus and with the use of a suitable scale-up criteria (flow rate/area of thin film evaporator), the industrial process with the condition of equal pressure (1-3 mbar) and temperature of thermostat 135 °C was dimensioned for 1 m^2 of heat transfer area with 12 l/h of incoming flow.

Priprava rekombinantne 20α-hidroksisteroid-dehidrogenaze (AKR1C1) in študije izražanja gena *AKR1C1* pri raku endometrija

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Rak endometrija je od hormonov odvisna bolezen, ki lahko nastane ob povečani koncentraciji estrogenov in zmanjšani koncentraciji progestagenov. Progesteron, aktivni progestagen, namreč nasprotuje mitogenemu delovanju estrogenov in vodi celice endometrija v diferenciacijo. Encimi, ki pretvarjajo progesteron v neaktivno obliko 20α-hidroksi-progesteron in obratno, so 20α-hidroksisteroid-dehidrogenaze (20α-HSD). Ti encimi so verjetno povezani z razvojem raka endometrija in predstavljajo potencialne tarče za razvoj novih zdravil. V okviru diplomske naloge smo proučevali AKR1C1, glavno 20α-HSD, ki spada v družino aldo-keto reduktaz. Rekombinantni človeški protein AKR1C1 smo pripravili v obliki fuzijskega proteina z glutation-S-transferazo v *E.coli* in očistili v eni stopnji z afinitetno kromatografijo. Določili smo substratno specifičnost encima ter K_m in V_{max} za progesteron. Nato smo ugotavljali, kako se gen AKR1C1 izraža v normalnem in rakavem tkivu endometrija iste osebe. Ugotovili smo, da je *AKR1C1* močneje izražen v petih vzorcih rakavega tkiva, pri treh vzorcih ni bilo bistvene razlike med normalnim in rakavim tkivom, pri enem samem vzorcu pa je bil gen *AKR1C1* močneje izražen v normalnem tkivu. Čeprav naši rezultati nakazujejo, da bi AKR1C1 lahko bil vpleten v nastanek te bolezni, bo v prihodnje hipotezo potrebno preveriti s študijami na večjem številu vzorcev. Vendar pa bodo naše raziskave pripomogle k razjasnitvi vloge AKR1C1 pri nastanku raka endometrija in pomena tega encima kot potencialne nove tarče za razvoj zdravil.

Preparation of Recombinant Human 20α-hydroxysteroid Dehydrogenase (AKR1C1) and Studies of *AKR1C1* Gene Expression in Endometrial Cancer

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Endometrial cancer is a hormone dependent disease caused by a high exposure to estrogens not opposed by progestagens. Estrogens increase the mitotic activity of endometrial cells and number of errors in DNA replication that can lead to malignant phenotype. Progesterone, the active progestagen has the opposite effect and drives cells into diferentiation. Enzymes that convert active progesteron into inactive 20α-hydroxy-progesterone and *vice versa* are 20α-hydroxysteroid dehydrogenases (20α-HSD). These enzymes may be involved in development of endometrial cancer and therefore represent potential targets for development of new drugs. We studied AKR1C1 the major 20α-HSD, a member of the aldo-keto reductase superfamily. Recombinant human enzyme AKR1C1 was prepared as a fusion protein with Glutathione-S-Transferase in *E.coli*. It was purified in one step using affinity chromatography. We determined substrate specifity, K_m and V_{max} for progesterone. Then we examined the level of *AKR1C1* expression in endometrial cancer and adjacent normal endometrium of the same patient. AKR1C1 expression was higher in five samples of endometrial cancer and almost unchanged in three samples. In one sample was AKR1C1 expression higher in normal endometrium than in endometrial cancer. Although our results indicate that AKR1C1 may be involved in development of endometrial cancer, more samples should be examined to confirm the proposed hypothesis. Our results will help to better understand the role of AKR1C1 in endometrial cancer and its importance as a putative drug target.

Vpliv sestave pelet izdelanih v rotorskem granulatorju na njihove izbrane tehnološke lastnosti

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V naših raziskavah smo proučili vpliv plastifikatorja na tehnološke lastnosti ogrodnih pelet izdelanih v rotorskem granulatorju. Za izdelavo pelet smo uporabili polivinilpirolidon kot tvornik ogrodja, glicerol kot plastifikator, vodo kot aglomeracijsko tekočino ter laktozo kot polnilo. Del polimera smo vključili v praškasto zmes, del pa v aglomeracijsko tekočino, v katero smo dodali še različne količine glicerola. Z uporabo glicerola se je zmanjšala krušljivost in izboljšala oblika in površina ogrodnih pelet.

The Influence of Pellets Composition Produced in Rotor Granulator on Their Selected Tehnological Characteristics

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In our research we have examined the influence of plasticizer on tehnological properties of matrix pellets produced in rotor granulator. In order to form pellets we have used polyvinylpyrrolidone as binder, glycerol as plasticizer, water as agglomeration liquid and lactose as filler. Part of polymer was included in powder mixture and other part in agglomeration liquid in which we also gave different amounts of glycerol. The friability of pellets has been reduced and shape and smoothness have been improved by the use of glycerol as plasticizer.

Priprava in karakterizacija koordinacijskih spojin molibdena(V) z acetatnim in formatnim ionom

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Oksomolibdenove spojine so prisotne tudi v aktivnih mestih nekaterih encimov (nitrogenaza, molibdopterinski encimi). Poznavanje kemije oksomolibdenovih spojin pomaga znanstvenikom pri razumevanju procesov, ki se odvijajo na teh mestih. Sinteza modelnih spojin je vodila v nepričakovan razcvet koordinacijske kemije molibdena v višjih oksidacijskih stanjih.
Proučevali smo vpliv reakcijskih pogojev na sintezo koordinacijskih spojin molibdena v oksidacijskem stanju +5 z acetatnim in formatnim ionom. Naši izhodni spojni sta bili (PyH)[$MoOCl_4(H_2O)$]•2/3PyHCl in $(PyH)_n[MoOBr_4]_n$. Običajno smo kot topilo uporabili acetonitril, v katerem smo raztopili izhodno spojino in ji dodali raztopino piridinijevega acetata ali formata. Reakcije smo izvajali tudi v zmeseh različnih topil: alkohola, piridina in acetonitrila. Nekatere reakcije so potekle že na sobni temperaturi, druge pri nižji temperaturi, v nekaterih primerih pa je bilo potrebno koncentriranje na vakuumski liniji. Tako smo s spreminjanjem razmerja med izhodno spojino in ligandom, pri različnih reakcijskih pogojih, dobili različne produkte.

Preparation and Characterization of Molybdenum(V) Coordination Compounds with Acetate and Formate Ions

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Oxomolybdenum species can be found in the active sites of certain enzymes, i.e., in nitrogenase and in a series of molybdopterin enzymes. The knowledge of the chemistry of the oxomolybdenum compounds could help scientists to understand the processes which are taking place at the enzymes' active sites. The syntheses of the model compounds resulted in an unexpected growth of molybdenum coordination chemistry, especially that with the metal in higher oxidation states.
One of the aims of the presented research was to study the influence of the reaction conditions over the formation of molybdenum(V) complexes either with acetate or formate ions. Mononuclear (PyH)[$MoOCl_4(H_2O)$]•2/3PyHCl or polymeric $(PyH)_n[MoOBr_4]_n$ were used as starting materials. The usual synthetic procedure involved the preparation of the acetonitrile solution of the starting material, followed by the addition of the stoichiometric amount of the ligand. In order to be able to add stoichiometric amounts of the ligand, the acetonitrile solutions of pyridinium acetate and pyridinium formate were prepared. In addition to the latter procedure, some of the reactions were also run in the mixture of more reactive solvents, normally the mixture consisted of pyridine, alcohol and acetonitrile. It was observed that some reactions took place at ambient conditions and some required elevated temperatures. Sometimes the removal of solvent under reduced pressure was required in order to isolate solid products. By changing the molar ratio between the ligand and the metal, different complexes were obtained.

Študij adsorpcije nekaterih kloriranih spojin na prst

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Klorirani pesticidi alaklor, lindan, heptaklor, DDE ter poliklorirana bifenila PCB-11 in PCB-138, ki ju v okolju najdemo kot posledico industrijskega onesnaženja, spadajo v skupino izredno toksičnih spojin. V svojem delu sem izbrane klorirane spojine ekstrahirala iz vodnih raztopin s pomočjo vlakna mikroekstrakcijske (SPME) igle. Po ekstrakciji sem spojine določala z metodo plinske kromatografije (GC) z detektorjem na zajetje elektronov (ECD).
Najprej sem določila optimalne pogoje za mikroekstrakcijo kloriranih spojin iz vodnih raztopin: čas mikroekstrakcije posameznih spojin iz vodnih raztopin, vpliv pH, detergenta in huminske kisline na mikroekstrakcijo spojin iz vodnih raztopin ter mejo zaznave, linearnost in ponovljivost SPME metode. SPME/GC-ECD metodo sem nato uporabila za študij adsorpcije izbranih kloriranih spojin iz vode na florisil, huminsko kislino, vrtno in gozdno prst. Pri študiju adsorpcije spojin na florisil, vrtno in gozdno prst sem ugotovila, da je bila adsorpcija spojin največja v prvem tednu eksperimenta. Po treh tednih so se bolj nepolarne spojine skoraj v celoti adsorbirale na florisil in prsti, majhna deleža bolj polarnih alaklora in lindana pa sta ostala prosta v vodi. Pri študiju adsorpcije kloriranih spojin na huminsko kislino pa je prišlo takoj po pripravi vzorca do interakcije preiskovanih spojin s huminsko kislino. SPME/GC-ECD analizo sem uporabila tudi za študij desorpcije kloriranih spojin s florisila, vrtne in gozdne prsti. Bolj nepolarne spojine so se ireverzibilno vezale na trdno fazo, polarnejša alaklor in lindan pa sta se po enem tednu desorbirala s florisila, vrtne in gozdne prsti.

Study of the Adsorption of Some Chlorinated Compounds on Soil

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The chlorinated pesticides alachlor, lindane, heptachlor, DDE and the polychlorinated biphenyls PCB-11 and PCB-138, which can be found in the environment as the consequence of industrial pollution, belong to a group of extremely toxic compounds. In this work some chlorinated compounds were extracted from aqueous solutions by the microextraction (SPME) fibre. After extraction the compounds were determined by gas chromatography (GC) with an electron-capture detector (ECD).
First the optimal conditions for a microextraction of chlorinated compounds from aqueous solutions were determined: the time needed for microextraction of each individual compound from aqueous solutions, the influence of pH, detergent and humic acid on a microextraction of compounds from aqueous solutions, the detection limit, linearity and repeatability of the SPME method.
SPME/GC-ECD method was later on used for studying the adsorption of chlorinated compounds from water on florisil, humic acid, garden and forest soil. The research of the adsorption of compounds on florisil, garden and forest soil showed that the adsorption was largest in the first week of experiment. After three weeks the less polar compounds were almost entirely adsorbed on florisil and soils, and small shares of more polar alachlor and lindane remained freely on the water. The research of the adsorption on humic acid have shown that immediately after preparing the sample, an interaction between the examined compounds and humic acid took place. SPME/GC-ECD analysis was also used for examination of the desorption of compounds from the florisil, garden and forest soil. The less polar compounds bound irreversible on the solid phase, while the more polar alachlor and lindane after a week desorbed from the florisil, garden and forest soil.

Farmakoekonomski vidiki pri zdravljenju artroze

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Artroza je zelo pogosta kronična revmatska bolezen, za katero je značilno prehitro in prezgodnje izginevanje sklepnega hrustanca. Degenerativne spremembe spremlja bolečina, okorelost in zmanjšana gibljivost sklepov. Od 45. do 54. leta je artroza pogostejša pri moških, po 54. letu pa pogostost artroze večja pri ženskah. Zdravljenje je simptomatsko in je zelo uspešno, če ga kombiniramo z nefarmakološkim zdravljenjem. Po priporočilih svetovnih revmatoloških združenj, se pri lažji obliki artroze uporablja za zdravljenje paracetamol, pri zmerni do hudi obliki pa se uporabljajo nesteroidna protivnetna in protirevmatična zdravila, ali pa kombinacija nesteroidnih protivnetnih in protirevmatičnih zdravil s tramadolom. Po svetovnih epidemioloških podatkih je prevalenca artroze 18%, kar pomeni, da je v Sloveniji približno 358.000 bolnikov z artrozo. Od tega jih ima lažjo obliko 232.700 (65%) bolnikov, 71.600 (20%) zmerno obliko in 53.700 (15%) hudo obliko artroze. V Sloveniji je poraba paracetamola manjša, poraba nesteroidnih protivnetnih in protirevmatičnih zdravil podobna, poraba tramadola pa je večja kot v razvitih državah. V Sloveniji se 50% vsega predpisanega paracetamola, 70% vseh predpisanih nesteroidnih protivnetnih in protirevmatičnih zdravil in 30% tramadola porabi za zdravljenje artroze. To znaša 1,63 DDD/1.000 prebivalcev/dan paracetamola, 32,15 DDD/1.000 prebivalcev/dan nesteroidnih protivnetnih in protirevmatičnih zdravil in 1,39 DDD/1.000 prebivalcev/dan tramadola v letu 2002. S pomočjo stroškovne analize smo na podlagi epidemioloških podatkov ocenili letne stroške zdravljenja. Skupni enoletni stroški optimalnega zdravljenja bi znašali 78.040,40 milijonov SIT.

Pharmacoeconomical Aspects of Treating Osteoarthritis

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Osteoarthritis is a very common chronic rheumatic disease. Degenerative changes are accompanied by pain and stiffness and loss of function. Below the age of 54, osteoarthritis is more common in men, but above the age of 55, it is more common in women. The management of osteoarthritis is symptomatic and comprises both pharmacological and non-pharmacological approaches. According to the Recommendations for the medical management of osteoarthritis, we use paracetamol for the treatment of mild osteoarthritis, while for treatment of moderate to severe osteoarthritis we use nonsteroidal anti-inflammatory drugs or combinations of nonsteroidal anti-inflammatory drugs with tramadol. The prevalence of osteoarthritis is 18%, which means that in Slovenia there are approximately 358.000 patients with osteoarthritis. Of these, 232.700 (65%) have mild osteoarthritis, 71.600 (20%) have moderate osteoarthritis and 53,700 (15%) have severe osteoarthritis. Slovenia has a lower use of paracetamol, a similar use of nonsteroidal anti-inflammatory drugs and a higher use of tramadol than other developed countries. In Slovenia 50% of all prescribed paracetamol, 70% of all prescribed nontsteroidal anti-inflammatory drugs and 30% of all prescribed tramadol is used for the treatment of osteoarthritis. This carries together, 1,63 DDD/1.000 inhabitants/day of paracetamol, 32,15 DDD/1.000 inhabitants/day of nonsteroidal anti-inflammatory drugs and 1,39 DDD/1.000 inhabitants/day of tramadol in 2002. Using cost-of-care analysis and epidemiological data we have estimated the costs for one year treatment. The cost for an optimal treatment of osteoarthritis was 78.040,40 million SIT.

Vrednotenje učinkovitosti vgrajevanja in stabilnosti ASKP v liposomih

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Namen raziskovalne naloge je bil izdelati sodoben nosilni sistem koloidnih velikosti za dostavo lipofilnega estra vitamina C-askorbilpalmitata (ASKP) v kožo, kjer naj bi deloval zaščitno pred prostimi radikali. ASKP smo vgradili v liposome, vodno disperzijo le-teh pa v hidrogel kot končno farmacevtsko obliko. V prvem delu raziskave smo proučili fizikalno in mikrobiološko stabilnost liposomov, sestavljenih iz nehidrogeniranega (NSL) in hidrogeniranega (HSL) sojinega lecitina ter homogeniziranih s sonikacijo in ekstruzijo. Kot merilo fizikalne stabilnosti smo s fotonsko korelacijsko spektroskopijo vrednotili velikost, polidisperzni indeks in zeta potencial liposomov. Na osnovi zgoraj omenjenih parametrov se je sonikacija izkazala za uspešnejšo metodo. Spremljali smo tudi vpliv temperature shranjevanja (sobna-pribl. 23 °C ali v hladilniku 8 °C) na fizikalno in mikrobiološko stabilnost. Ugotovili smo, da na fizikalno stabilnost liposomov temperatura ne vpliva, medtem ko je bila mikrobiološka stabilnost bistveno boljša pri vzorcih shranjenih v hladilniku. Slednjo smo še izboljšali z dodatkom konzervansov, ki niso imeli vpliva na lastnosti liposomov. V drugem delu raziskave smo v liposome vgradili ASKP, zaradi česar so ti postali večji, kljub temu pa fizikalno stabilni. Učinkovitost vgrajevanja ASKP v liposome smo določili s HPLC/UV metodo po dializi in ugotovili, da se je največji delež ASKP vgradil v sonicirane NSL liposome. Slednji tudi najbolj zaščitijo ASKP pred razpadom, kar smo kvantitativno spremljali 42 dni. Na koncu smo vodno disperzijo liposomov vgradili v hidrogele štirih različnih sestav in glede na izmerjene reološke lastnosti kot optimalnega izbrali hidroksietilcelulozni gel. Zaključimo lahko, da so proučevani liposomi z vidika lastne stabilnosti ustrezen nosilni sistem, medtem ko ne zagotavljajo ustrezne stabilnosti ASKP, zato bi bilo smiselno raziskave nadaljevati predvsem v smeri povečevanja le-te.

Entrapment Efficacy and Stability of Ascorbylpalmitate in Liposomes

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The goal of our research was development of a modern carrier system for the delivery of lipophilic derivative of vitamin C, ascorbylpalmitate (ASKP) into the skin to protect it from free radicals. ASKP was incorporated in the liposomes and the water dispersion of them in a hydrogel as a dosage form. The first part of our research focused on the evaluation of physical and microbiological stability of liposomes, consisted of hydrogenated and nonhydrogenated soy lecithin and homogenized by sonication and extrusion. As parameters of physical stability size, polydisperse index and zeta potential with photonic correlation spectroscopy (PCS) was evaluated. On the basis of above-mentioned parameters sonication was proved as more suitable method. We monitored also the effect of storage temperature (at room conditions-23 °C and in cold storage 8 °C) on physical and microbiological stability. The temperature did not affect the physical stability, but the samples kept in the refrigerator were more microbiologically stable. The latter was further enhanced by adding preservatives which did not affect liposomes properties. In the second part of our work ASKP was loaded in liposomes, which increased their size but did not reduce physical stability. The entrapment efficacy was determined by HPLC/UV method after dialysis. The best entrapment efficacy and ASKP stability was achieved for sonicated NSL liposomes. In the last part of study we loaded the liposomes with ASKP in 4 different hydrogels. Hidroxyethylcelullose gel demonstrated the most appropriate reological properties. We can conclude, that liposomes are a suitable delivery system, but further investigation should be focused on increasing the ASKP stability in liposomes.

Primerjava moči antagonista receptorjev H_2 na izoliranem želodcu po Schildovi in Calderonejevi metodi

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Najbolj zanesljivo in največkrat preizkušeno Schildovo metodo za ugotavljanje moči in narave kompetitivnih antagonistov smo uporabili kot referenčno metodo za ugotavljanje primernosti novejše, Calderonejeve metode za določevanje moči antagonista nizatidina na izoliranem želodcu budre z agonistom histaminom, ki ob vezavi na histaminske receptorje H_2 vzpodbudi izločanje želodčne kisline in antagonistom nizatidinom, ki zmanjša učinek histamina. Po obeh metodah smo poskusili ugotoviti tudi naravo nizatidina. Schildova metoda nam je nepresegljivo naravo nizatidina razkrila, Calderonejeva metoda pa ne. Po Schildovi metodi smo določili, da je vrednost za moč nizatidina 6.01, s 95% mejami zaupanja med 4.59 in 13.29, in z vrednostjo naklona regresijske premice 0.91 s 95% mejami zaupanja med 0.17 in 1.65 in statistično značilno ni različen od ena, zato ne moremo ovreči, da je nizatidin kompetitiven antagonist. Po Calderonejevi metodi pa je vrednost 6.88, vendar meja zaupanja statistično ni bilo mogoče določiti. Naklon, določen s Calderonejevo metodo pa je 0,46 in ima 95% meje zaupanja med 0,46 in 1,37. Tudi ta naklon statistično značilno ni različen od ena, prav tako se vrednost naklona po obeh analizah statistično značilno ne razlikuje med sabo. Sklepamo lahko, da je vrednost za moč nizatidina po Schildovi in Calderonejevi metodi na izoliranem želodcu budre enaka.

The Comparison of the Potency of an H_2 Receptor Antagonist on the Isolated Stomach of Guinea Pig by Use of the Methods According to Schild and According to Calderone

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The most reliable and most frequently used method for determining the potency and the nature of competitive antagonists is the Schild method, which we used as a reference to ascertain the adequacy of the newer, Calderone method for establishing the potency of antagonists on isolated stomach of a guinea pig. The secretion of acid from parietal cells was provoked by adding histamine, an efficient agonist and we reduced its activity by the presence of competitive antagonist nizatidine. Both methods were also used to define the nature of nizatidine. The Schild method revealed the insurmountable nature of nizatidine, whereas Calderone method did not. With the results of the Schild method it was determined that the value of potency of nizatidine was 6.01, with 95% limits of confidence between 4.59 and 13.29 and the slope of regression line has the value of 0.91 with 95% limits of confidence between 0.17 and 1.65 and is statistically significantly not different from unity, therefore it can be concluded that nizatidine is a competitive antagonist. The potency after Calderone method is 6.88, but limits of confidence could not be statistically determined, and the slope is 0.46 and has 95% limits of confidence between 0.46 and 1.37. To conclude, the values of potency of nizatidine determined following both the Schild and the Calderone methods on the isolated stomach of a guinea pig are the same.

Hidroliza karboksimetil celuloze (CMC), katalizirana z imobilizirano celulazo iz *Humicola insolens*

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Delo predstavlja rezultate raziskav encimsko katalizirane hidrolize karboksimetil celuloze (CMC). CMC je topna v vodi in ima širok spekter uporabe (v tekstilni, papirni, prehrambeni industriji ter v industriji detergentov). Kot katalizator smo pri vseh reakcijah uporabili celulazo, imobilizirano na aerogel. Z zamreženjem encima v sol – gel matriko (aerogel) smo preprečili izpiranje encima, zmanjšali denaturacijo le – tega ter omogočili, da smo encim lahko uporabili večkrat.
Reakcijo hidrolize smo izvajali v dveh vrstah reaktorjev: v mešalnem šaržnem reaktorju in v cevnem pretočnem reaktorju z nasutim slojem biokatalizatorja. Želeli smo določiti optimalne pogoje za reakcijo hidrolize CM celuloze. Zato smo študirali vplive reakcijskih parametrov (koncentracije biokatalizatorja, koncentracije substrata, temperature, pH) na kinetiko omenjene reakcije. Prav tako smo želeli določiti razpolovni čas imobilizirane celulaze ter stabilnost celulaze (imobilizirane in proste) v superkritičnem ogljikovem dioksidu. V cevnem pretočnem reaktorju z nasutim slojem biokatalizatorja pa smo želeli proučiti vpliv pretoka substrata na presnovo.

Hydrolysis of Carboxymethyl Cellulose (CMC), Catalysed by Immobilized Cellulase from *Humicola insolens*

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A study of enzyme catalysed hydrolysis of carboxymethyl cellulose (CMC) is presented in this work. CMC is soluble in water and has a wide range of usage (in textile, paper, food and detergent industry). For all reactions the enzyme cellulase, immobilized on aerogel, was used as a catalyst. The meshing of the enzyme in a sol – gel matrix (aerogel) prevented the enzyme from washing out, the enzyme could be reused many times and the denaturation of the enzyme could be also reduced.
The reaction of hydrolysis was performed in two different types of reactors: in a batch – stirred tank reactor and in a packed – bed continuous – operated reactor. Because of the determination of optimal reaction conditions for enzymatic hydrolysis of carboxymethyl cellulose, the influence of reaction parameters (biocatalyst concentration, substrate concentration, temperature, pH) on the kinetics of enzyme catalysis reaction was studied. The half – life for enzyme and the stability of cellulase (immobilized and crude) in supercritical carbon dioxide was studied, as well. The influence of substrate flux on the conversion was studied in packed – bed continuous – operated reactor.

Proučevanje vpliva parametrov izdelave trdnih disperzij na hitrost raztapljanja nifedipina

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Biološko uporabnost slabo topnih zdravilnih učinkovin lahko izboljšamo s pripravo farmacevtskih oblik s hitrim raztapljanjem kot so trdne disperzije. S preskusi raztapljanja po USP XXVI smo ugotovili, da pripravljene trdne disperzije nifedipina s polietilen glikolom (PEG) 4000 oziroma manitolom kot nosilcema bistveno izboljšajo hitrost raztapljanja v primerjavi z fizikalnimi zmesmi iste sestave ter čistim nifedipinom. PEG 4000 je ustreznejši nosilec v smislu povečanja hitrosti raztapljanja nifedipina v primerjavi z manitolom, prav tako večji masni delež nosilca izboljša raztapljanje. Hitrost ohlajanja taline trdne disperzije vpliva na hitrost raztapljanja, vendar se v našem primeru ni izkazala za kritičen parameter, saj kasnejša mehanska obdelava razlike zabriše oziroma spremeni. Možno je tudi, da so vse uporabljene hitrosti ohlajanja dovolj velike, da povzročijo nastanek strukture iste stopnje urejenosti. Pri določenih sistemih trdnih disperzij, kjer ima nosilec bistveno nižjo točko tališča (PEG) od zdravilne učinkovine in gre za z nosilcem kontrolirano hitrost raztapljanja, bi bile možne in prednostne modifikacije metode taljenja v smislu dispergiranja praška učinkovine v talino nosilca.

A Study of Influence of Solid Dispersions Manufacturing Parameters on the Dissolution Rate of Nifedipine

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Bioavailability of poorly water-soluble drugs can be improved with designing dosage forms with enhanced dissolution like solid dispersions. Using dissolution tests of USP XXVI we came to the conclusion that nifedipine-polyethylene glycol (PEG) 4000 or nifedipine-mannitol solid dispersions essentially improve dissolution rate with regard to the prepared physical mixtures of the same composition and pure nifedipine. PEG 4000 is a more suitable carrier in order to enhance dissolution rate of nifedipine compared to mannitol. At the same time a greater mass part of a carrier enhances the dissolution. Cooling rate of solid dispersion melt affects dissolution rate though we did not find it a critical parameter. The mechanical stress applied by grinding changes or blurs the differences. It is also possible that all used cooling rates are sufficient enough to cause a formation of a structure with the same degree of order. In some systems of solid dispersions, where a carrier has a substantially lower melting point (PEG) than a drug itself, and a carrier-controlled dissolution is proposed, we could take the advantage of some feasible modifications of melting method. Thus it would be possible to disperse powder of a drug in to a melt of a carrier.

Razvoj standarda kakovosti na podlagi GMP v Krki

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Dobra proizvodna praksa je del sistema za doseganje kakovosti, ki v proizvodnji in kontroli proizvodnje zdravil določa pogoje za izdelavo zdravila v skladu s specifikacijo kakovosti izdelka, zapisanega v dovoljenju za promet. Srečamo jo v različnih vrstah proizvodenj, saj temelji na pozitivnih izkušnjah v organizaciji ter izvedbi proizvodnih in kontrolnih nalog, ki prinašajo dobre rezultate v zagotavljanju kakovosti izdelkov. DPP je interes in notranja zahteva vsakega proizvajalca, ki hoče na trg plasirati kakovostne in konkurenčne izdelke. V Krki proizvajajo izdelke posebnega pomena, gre namreč za izdelke, ki se uporabljajo za zdravljenje in preprečevanje bolezni v humani in veterinarski medicini. Zato je tudi zahteva po upoštevanju in izvajanju proizvodnje in kontrole v skladu z zahtevami dobre proizvodne prakse toliko strožja.

Standard Quality Development Based on GMP in Krka

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Good Manufacturing Practice is a part of system for achieving quality that in the process and control of pharmaceutical production determines conditions for manufacturing of medicines in compliance with specifications for the quality of the product as recorded in its marketing authorisation. GMP is present in different types of industries and is based on the positive results achieved in the organization, and on such production and control processes that provide good results in view of quality assurance of the products. Every manufacturer who has the intention of placing quality and competitive products on the market is interested in providing GMP, and regards it as an internal standard. Krka is a manufacturer of products with special value. These are products used for the treatment and prevention of diseases in human and veterinary medicine. In view of this, the directives for following and conducting the production and control in concordance with the GMP requirements are even stricter than in other industries.

Kvantitativni odnos med strukturo in delovanjem (QSAR) peptidomimetikov s 3,4-dihidro-2*H*-1,4-benzoksazinskim skeletom

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Hemostaza ima pomembno vlogo pri ohranjanju integritete krvožilnega sistema. Posledica patoloških sprememb so različni kardiovaskularni zapleti, ki predstavljajo glavni vzrok smrti v razvitem svetu. Obetavne zdravilne učinkovine predstavljajo antagonisti fibrinogenskega GP IIb/IIIa receptorja in inhibitorji trombina. Izvedli smo QSAR študije za molekule s 3,4-dihidro-2*H*-1,4-benzoksazinskim skeletom. Na podlagi 3D strukture smo s programom CODESSA izračunali velik nabor molekulskih deskriptorjev in konstruirali več QSAR modelov za obe tarči. Dobljenim QSAR modelom smo ovrednotili napovedne sposobnosti, koeficiente najboljšega QSAR modela za posamezno tarčo pa ortogonalizirali z Randićevo metodo. Analizirali smo posamezne molekulske deskriptorje ter sklepali na strukturne zahteve pomembne za vezavo na izbrani tarči. Izvedli smo molekulsko sidranje v aktivno mesto trombina s programom FlexX ter afiniteto dobljenih kompleksov ovrednotili z empiričnimi cenilnimi funkcijami. Dobljene napovedi smo primerjali z napovedmi, pridobljenimi s statistično analizo v programu CODESSA. Ugotovili smo, da slednja daje boljše rezultate, saj napovedi generira na osnovi poznavanja biološke aktivnosti. Z uporabo molekulske grafike smo ovrednotili izbrani kompleks inhibitor-trombin. Pregled strukture kompleksa jasno odraža pomen hidrofobnih interakcij in potrjuje, da so molekulski deskriptorji odraz dejanskih vezavnih karakteristik tega tipa molekul.

Quantitative Structure Activity Relationship (QSAR) of Peptidomimetic Agents with 3,4-Dihydro-2*H*-1,4-benzoxazine Scaffold

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Homeostasis plays an important role in maintaining normal function of cardiovascular system. As a consequence of pathological changes cardiovascular abnormalities may occur. These pathological states represent main causes of illnesses and mortality in developed countries. As promising new active molecules fibrinogen GP IIb/IIIa receptor antagonists and thrombin inhibitors were developed. In this work QSAR studies were performed for selected molecules all possessing 3,4-dihydro-2*H*-1,4-benzoxazine scaffold. Based on calculated 3D structure a large number of molecular descriptors was calculated within CODESSA program. Several QSAR models for both selected macromolecular targets were developed and analyzed. The coefficients of the best two QSAR equations were ortogonalised by Randić method. Interpretation of these two QSAR models was performed by examining molecular descriptors and structural characteristics important for successful binding were elucidated for both molecular systems. Molecular docking for thrombin inhibitors was performed by using FlexX. The affinity of obtained complexes was evaluated by empirical scoring functions. Results of these predictions were compared with those obtained from CODESSA statistical analysis. It was established that CODESSA predictions were significantly superior to those obtained with scoring functions due to the weakness of the scoring functions in ranking of molecular binding energy. Interpretation of the structure of a selected complex thrombin- inhibitor was done by using molecular graphics. The complex structure indicates the importance of hydrophobic interactions thus shows the ability of molecular descriptors to reflect the structural characteristics important for successful binding.

Analiza polimorfizmov v promotorju in intronu 2 gena za serotoninski prenašalec pri samomorilcih

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V Sloveniji si vsako leto vzame življenje okoli 600 posameznikov, zaradi česar je samomor postal zaskrbljujoč nacionalni problem. Ravno visok samomorilni količnik (okoli 30 samomorov na 100.000 prebivalcev) pa je vzrok aktualnosti preučevanja samomorilnosti v slovenskem prostoru.
Študije samomorilnosti so se osredotočile predvsem na serotoninski sistem, kateremu pripisujejo pomembnejšo vlogo pri uravnavanju razpoloženja, impulzivnosti in agresivnosti – lastnostih, povezanimi s samomorilnim vedenjem. Raven serotonina v organizmu uravnavajo biosintezni, metabolni in transportni proteini. Ključno vlogo pri uravnavanju aktivnosti serotonina v sinapsi ima serotoninski prenašalec. Navzočnost polimorfizmov v genu tega proteina lahko spremeni njegovo izraženost ali strukturo samega serotoninskega prenašalca. Posledica omenjenih sprememb se lahko kaže v spremenjeni aktivnosti prenašalca in ravni serotonina.
V nalogi smo pri vzorcih DNA 102 samomorilcev in 138 kontrolnih oseb analizirali funkcionalna polimorfizma insercije/delecije 44 bp v promotorju (5-HTTLPR) in spremenljivega števila tandemskih ponovitev 17 bp (VNTR) v intronu 2 gena za serotoninski prenašalec.
Polimorfne regije DNA smo namnožili z reakcijo PCR in jih analizirali z agarozno gelsko elektroforezo. Statistična obdelava dobljenih rezultatov ni pokazala signifikantne razlike v alelnih in genotipskih frekvencah med populacijama samomorilcev in kontrolnih oseb in s tem tudi ne povezave omenjenih polimorfizmov s samomorilnostjo.

Analysis of Polymorphisms in the Promoter and Intron 2 of the Serotonin Transporter Gene in Suicide Victims

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In Slovenia 600 individuals commit suicide every year and this is considered to be an alarming national problem. High suicide rate (about 30 suicides per 100.000 inhabitants per year) is the reason behind the high interest in suicide studies on Slovenian ground.
Suicide studies are focused mainly on serotonergic system, which has an important part in dealing with balancing disposition, impulsiveness and aggression – characteristics typical for suicidal behavior. Biosynthetic, metabolic and transporting proteins are regulating the serotonin level in the organism. The serotonin transporter plays a central role in regulating the level of activity in serotonin synaptic function. Presence of polymorphisms on the protein's gene can change the expression of the gene itself or the structure of serotonin transporter. Consequences can be seen in the changed transporter activity or in the level of serotonin.
In our study we analysed two functional polymorphisms in the promoter (insertion/deletion 44 bp – 5-HTTLPR) and in the second intron (variable number of tandem repeats 17 bp – VNTR) of the serotonin transporter gene on DNA samples of 102 suicide victims and 138 healthy control subjects.
Both polymorphic regions were amplified by polymerase chain reaction (PCR) and PCR products were analysed by agarose gel electrophoresis. Statistical processing of experimental results showed no significant differences and 5-HTTLPR in intron 2 VNTR genotype neither in allele frequency distribution between suicide victims and healthy control subject suggesting that there is no association between studied polymorphisms and suicide behavior.

Citotoksični vpliv različnih platinskih(II) kompleksov na različne vrste tumorskih celic

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Citostatiki CDDP (cisplatin), OKSA (oksaliplatin) in KARBO (karboplatin) so platinski(II) kompleksi, ki se že uporabljajo v kliniki. Zaradi toksičnosti za normalna tkiva, razvoja odpornosti in stranskih učinkov se sintetizirajo novi kompleksi. Z našimi raziskavami smo v *in vitro* pogojih določali citotoksičnost novih platinskih(II) kompleksov (3P-SK, Pt, 3P-M, 3P-CB), ki so bili sintetizirani na Fakulteti za kemijo in kemijsko tehnologijo Univerze v Ljubljani, v primerjavi s CDDP, OKSA in KARBO na štirih različnih humanih tumorskih celicah (karcinomske celice mehurja T24, karcinomske celice ovarija IGROV1 in njihove neobčutljive hčerinske celice IGROV1/RDDP ter karcinomske celice dojke MCF7). Celice smo nasadili na mikrotiterske plošče, dodali citostatike in vse skupaj inkubirali 3 oziroma 5 dni na 37ºC v atmosferi s 5% CO_2. Citotoksičnost citostatikov smo določevali z MTT testom. Za vsako celično linijo smo določili inhibitorno vrednost IK_{50}, ki predstavlja koncentracijo citostatika, pri kateri preživi 50% celic. Citostatika CDDP in OKSA sta bila najbolj citotoksična na vseh testiranih celicah. Novi platinski(II) kompleks 3P-SK je bil bolj citotoksičen kot KARBO, na vseh testiranih celicah, razen na MCF7. Ostali platinski(II) kompleksi so bili manj citotoksični. Med vsemi celicami, ki smo jih uporabili v poskusih, so bile celice IGROV1 najbolj občutljive, njihove hčerinske celice IGROV1/RDDP pa najmanj. Celice T24 in MCF7 so bile občutljive na CDDP, OKSA in 3P-SK, na KARBO ter ostale platinske(II) komplekse Pt, 3P-M in 3P-CB pa so bile neobčutljive. Zaradi citotoksičnosti je platinski(II) kompleks 3P-SK, primeren kandidat za nadaljnje raziskave *in vivo*.

Cytotoxicity of Different Platinum(II) Complexes on Different Human Tumor Cell Lines

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CDDP (cisplatin), OKSA (oxaliplatin) and KARBO (carboplatin) are platinum(II) complexes that are already used in the clinical practice. Because of their side effects (toxicity, development of resistance) new platinum(II) complexes are being syntehesized. The aim of our study was to determine *in vitro* cytotoxic activity of four new platinum(II) complexes (3P-SK, Pt, 3P-M and 3P-CB) in comparison with CDDP, OKSA and KARBO on four different human tumor cell lines (bladder carcinoma T24, ovarian carcinoma IGROV1, ovarian resistant IGROV1/RDDP carcinoma and mammary carcinoma MCF7 cells). Cells were plated into 96-well microtiter plates and incubated with platinum complexes for 3 or 5 days at 37ºC in humidified atmosphere containing 5% CO_2. Cytotoxicity of the complexes was determined by the colorimetric MTT assay. The inhibitory concentration of the drug that reduced survival of cells to 50% (IK_{50}) was determined for each cell line. The results of our study showed that CDDP and OKSA were the most effective in reducing cell survival on all cells used. One of the new platinum(II) complexes, 3P-SK, was more cytotoxic compared to KARBO on all cells used, except on MCF7. The most sensitive cells to all complexes, as determined by IK_{50}, were human ovarian carcinoma IGROV1. Cells IGROV1/RDDP were the most resistant. Cell line T24 and MCF7 were sensitive to CDDP, OKSA, and 3P-SK, but they were resistant to KARBO, Pt, 3P-M and 3P-CB. Platinum(II) komplex 3P-SK showed high cytotoxic effect against all cell lines tested, so further *in vivo* studies are warranted.

Primerjava bakterijske in bakteriofagne peptidno predstavitvene knjižnice pri iskanju peptidnih motivov z afiniteto do streptavidina

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Biološke kombinatorične peptidne knjižnice so sestavljene iz velikega števila (do 10^9 neodvisnih klonov) naključnih peptidov, ki so največkrat predstavljeni na površini bakteriofagov ali bakterij. Z zmogljivim rešetanjem, lahko iz knjižnice selekcioniramo klone, ki nosijo peptide z visoko afiniteto do tarčne molekule. Odkriti peptidni ligandi predstavljajo začetno točko v odkrivanju novih zdravilnih učinkovin. Objavljenih je bilo že veliko člankov o uspešnih selekcijah z bakteriofagno knjižnico in le nekaj člankov o uspešnih selekcijah z bakterijsko knjižnico. Ta dva sistema do sedaj eksperimentalno še niso primerjali, zato smo se v naši raziskovalni nalogi odločili za primerjavo bakterijske peptidno predstavitvene knjižnice FliTrx™ in bakteriofagne peptidno predstavitvene knjižnice Ph.D C7C™ v sposobnosti selekcije peptidov z afiniteto do streptavidina. V peptidnih sekvencah selekcioniranih klonov smo iskali znani peptidni ligand za streptavidin (His/Pro/Gln motiv) in na podlagi prisotnosti tega motiva primerjali učinkovitost obeh metod. Glede na naše rezultate klasične selekcije z bakterijsko in bakteriofagno knjižnico, se je bakteriofagna peptidno predstavitvena knjižnica izkazala kot primernejša metoda za iskanje novih peptidnih ligandov. Menimo, da bi z izpopolnjenim postopkom selekcije, učinkovita metoda lahko postala tudi uporaba bakterijskih knjižnic.

Comparison of Bacterial and Bacteriophage Peptide Display Library in Selection of Streptavidin Binding Peptides

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Biological combinatorial peptide libraries consists of vast number of random peptides (up to 10^9 independent clones), most often displayed on the surface of bacteriophages or bacteria. With high-troughput screening of the library, one can select clones bearing a peptide with high affinity to target molecule. New peptide ligands may lead to the discovery of a new active pharmaceutical ingredients. There are many articles already published on successful selections with phage display, but only few on successful selections with bacterial display. These two techniques have never been experimentally compared before. In this work, we compared bacterial peptide display library FliTrx™ and bacteriophage peptide display library Ph.D C7C™ in selection of streptavidin binding peptides. Comparison of the effectiveness of the two methods was based on the presence of already known streptavidin peptide ligand (His/Pro/Gln motiv) in peptide sequence of selected clones. According to our results of classic selection with bacterial and bacteriophage library, we found bacteriophage peptide display library to be more efficient method for the discovery of new peptide ligands. In our opinion, the bacterial library could also become an efficient method, if selection procedure is improved.

Vpliv koncentracije in vrste železove spojine na akumulacijo železa v biomasi kvasovke *Saccharomyces cerevisiae*

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Anorganski dodatki železovih spojin v prehrano so slabo izkoristljivi, lahko so celo toksični. Novo in varnejšo rešitev lahko predstavlja kvasna biomasa, obogatena z železom. V nalogi smo preučevali vpliv koncentracije in vrste železovih spojin v tekočem gojišču na rast in razmnoževanje, akumulacijo železa in kultivabilnost kvasovke *S. cerevisiae*. Količino akumuliranega železa (Fe) v kvasni biomasi po 24-urni kultivaciji smo določali z atomsko absorpcijsko spektroskopijo. Kultivabilnost je število kolonijskih enot/ml glede na skupno število celic/ml po končani kultivaciji. Relativni odstotek kultivabilnosti je izračunan glede na kontrolni poskus. Odsotnost Fe iz gojišča je negativno vplivala na rast in razmnoževanje kvasovk. Relativne kultivabilnosti kvasovk so bile pri dodatku izbranih železovih spojin v gojišče do koncentracije 100 µM oziroma 1 mM (Fe(III) citrat) nižje od 100%. Količina akumuliranega Fe v kvasni biomasi je bila pri dodatku Fe(III) citrata, $FeCl_3 \cdot 6\ H_2O$, $Fe(NO_3)_3 \cdot 9\ H_2O$ v koncentracijskem območju do 100 µM v gojišče neodvisna od vrste železove spojine, a je naraščala sorazmerno s koncentracijo Fe v gojišču. Preučevane koncentracije kompleksa Fe-EDTA v gojišču (10–100 µM) niso vplivale na količino akumuliranega Fe v kvasni biomasi. Maksimalna akumulacija, 13 µg Fe/mg suhe biomase, je bila dosežena pri 1 mM koncentraciji Fe(III) citrata v gojišču. Rezultati so pokazali, da je z naraščanjem koncetracije Fe(III) citrata v gojišču (10–100 mM) upadala količina akumuliranega Fe v kvasni biomasi.

The Effect of Iron Compound Concentration and Iron Compound Type on Iron Accumulation in the Biomass of the Yeast *Saccharomyces cerevisiae*

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Inorganic iron food supplements are less well absorbed and can be even toxic. The yeast biomass enriched with iron could represent a new and safer solution. Effects of iron compounds type and iron compounds concentration in the medium on the yeast growth and proliferation, iron accumulation and cultivability of yeast *S. cerevisiae* were studied. The amount of accumulated iron (Fe) in the yeast biomass after 24h cultivation was determined with atomic absorption spectrometry. The yeast cultivability is CFU/ml with regard to total cell number/ml after the 24h cultivation. The relative cultivability percentage was calculated with regard to the control experiment. The absence of Fe in the growth medium inhibited the growth and proliferation of yeasts. At addition of iron compounds up to 100 µM or 1 mM (Fe(III) citrate) in the growth medium the relative cultivabilities of yeasts were lower than 100%. The amount of accumulated Fe in the yeast biomass cultivated in the growth medium with added Fe(III) citrate, $FeCl_3 \cdot 6\ H_2O$, $Fe(NO_3)_3 \cdot 9\ H_2O$ up to 100 µM was independent of the iron compound type, but increased with the iron compound concentration in the medium. Investigated Fe-EDTA concentrations in the medium (10–100 µM) did not affect the iron accumulation in the biomass. The maximum amount of accumulated iron, 13 µg Fe/mg dry biomass, was reached at 1 mM Fe(III) citrate in the growth medium. The results showed, that with the increasing Fe(III) citrate concentration in the medium (10–100 mM), the amount of accumulated iron in the yeast biomass decreased.

Vpliv zamreženja in polimerizacijskih pogojev na fizikalno – kemijske lastnosti akrilatnih polimernih nosilcev

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Z vodno suspenzijsko polimerizacijo smo sintetizirali netopne kopoli(stiren-4-nitrofenilakrilatne) nosilce s pet in dvajset odstotnim zamreženjem ter različnim razmerjem akrilat/stiren (1/1, 2/1 in 1/0). Sintezo smo izvedli v 0,5 L posodi ob uporabi teflonskega mešala pri 750 min^{-1}. S prosto radikalsko polimerizacijo smo dobili polimerne nosilce v obliki kroglic premera med 10 in 90 µm. Višja stopnja zamreženja in zamreženje z divinilbenzenom dajeta delce z večjim premerom. Pet odstotno zamreženi nosilci v metanolu, vodi, acetonitrilu, diklorometanu in toluenu nabrekajo bolj kot dvajset odstotno zamreženi. Gibljivost in reaktivnost polimerov smo študirali z modelnimi reakcijami z 1,8-diaminooktanom. Pri pretvorbi sta možna dva tipa produkta; razmerje med njima smo določili z analizo dušika in ugotovili, da je razmerje produktov pri nosilcih zamreženih z divinilbenzenom neodvisna od odstotka zamreženosti, s povečevanjem deleža 4-nitrofenilakrilata pa se povečuje prisotnost dodatno zamreženega produkta. Pri nosilcih zamreženih z N,N-etilenglikoldimetakrilatom je razmerje med nastalima produktoma odvisno od deleža 4-nitrofenilakrilata in stopnje zamreženosti. Nosilce poli(akrilne kisine) smo sintetizirali z inverzno suspenzijsko polimerizacijo, z uporabo N,N-metilenbisakrilamida kot zamreževala in amonijevega persulfata kot iniciatorja. Z etil celulozo smo pripravili pet tipov nosilcev pri različnih hitrostih mešanja in količini stabilizatorja ter ugotovili linearno koleracijo med hitrostjo mešanja in povprečnim premerom delcev. Nabrekanje nosilcev poli(akrilne kisline) smo določili v vodi, metanolu, acetonitrilu, diklorometanu in toluenu ter ugotovili, da polimerni nosilci v prvih dveh topilih nabrekajo precej bolj kot v zadnjih treh. Stopnja nabrekanja različnih tipov nosilcev je v posameznem topilu skoraj identična. Poli(akrilno kislino) smo pretvorili v poli(akriloil klorid) in analizirali časovni potek reakcije.

Effect of Crosslinking and Polymerisation Conditions on the Physical- Chemical Properties of Acrylate Polymer Supports

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Unsoluble crosslinked poly(styrene-4-nitrophenylacrylate) polymer resins with 5% and 20% of crosslinker and various ratios of styrene to acrylate were prepared using free radical polymerisation. Spherical polymer supports between 10 and 90 µm were obtained by water suspension polymerisation using PTFE paddle shaped stirrer at 750 rpm. Higher crosslinking level and divinylbenzene gave particles with larger diameters. Polymers with lower crosslinking density swell in methanol, water, acetonitrile, dichloromethane and toluene to a bigger extent than polymers with higher crosslinking level. Flexibilities of polymer matrixes were studied using reactions with 1,8-diaminooctane as a model substrate. Level of additional crosslinking was determined by combustion analysis of nitrogen. We found that higher acrylate content resulted in a higher level of additional crosslinking. Furthermore, in the case of polymers crosslinked with ethyleneglycoldimethacrylate, additional crosslinking also depended on the initial crosslinking level. Crosslinked poly(acrylic acid) using N,N-methylenebisacrylamide as a crosslinker was also prepared using suspension polymerisation technique. Stirrer speed and the amount of the stabiliser varied and a liner corelation between the stirrer speed and an average particle diameter was found. Swelling capacity of these supports in water, methanole, acetonitrile, dichloromethane and toluene was studied and a higher capacity in methanol and water was found. An immobilised acid chloride was prepared from poly(acrylic acid) and the velocity of the transformation monitored by the determination of chlorine content and FTIR spectroscopy.

Določevanje čajnih mešanic na osnovi nukleotidnih zaporedij ITS regij s pomočjo restrikcijske analize

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Čeprav so danes v ospredju predvsem sintezna zdravila, pa je uporaba tradicionalnih fitoterapevtikov še vedno aktualna. Tako danes zasledimo prihod novejše, racionalne fitoterapije, kjer pa so zdravilni čaji kljub svoji »tradicionalnosti« še vedno ena najpogostejših izbir pri uporabi zdravilnih rastlin za samozdravljenje najrazličnejših obolenj. Na trgu je mogoče dobiti širok spekter drog kot tudi njihovih potvorb, zato je tudi spekter delovanja le teh in s tem možnost nastanka neželenih učinkov pri nenadzorovani porabi še toliko večji. To zahteva zanesljive in natančne tehnike določevanja kakovosti.
Z razvojem vse sodobnejših tehnologij je možno le te uporabiti tudi pri določevanju kompleksnih mešanic, pri tem čaji niso nikakršna izjema. V dani raziskovalni nalogi smo na podlagi izoliranih vrstno specifičnih ITS regije, ki se nahajajo v genskem zapisu za rDNA, razvili postopek za določanje in razlikovanje večkomponentnih mešanic drog.

Determination of Herbal Tea Mixtures on the Basis of ITS Regions Nucleotide Sequences Using Restriction Analysis

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Although nowadays synthetic drugs are more at the fore, the use of traditional phytotherapeutics is still very a live. Today we are witnessing the coming of newer, rational phytotherapy, where herbal teas in spite of their traditionality are still one of the most frequent choices in the usage of medicinals herbs for self-treatment of different health problems. The market is offering a wide spectrum of medicinals plants as well as their fakes, therefore their activity spectrum and the possibility of the occurrence of unwanted side-effects with the uncontrolled usage is considerable. This requires reliable and accurate techniques of quality control. The development of modern technologies can be applied for the determination of complex mixtures and herbal teas are no exceptions. In our research we developed a method for determination and differentiation of multicomponent tea mixtures on the basis of isolated, species-specific ITS regions that are found in the genetic code of rDNA.

Pridobitev Fab fragmenta visoko avidnih imunoglobulinov razreda G proti beta-2-glikoproteinu I

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Protitelesa proti beta-2-glikoproteinu I so glavna podskupina protiteles, ki se pojavljajo pri bolnikih z avtoimunskim obolenjem, imenovanim antifosfolipidni sindrom. Čeprav so od vseh kazalcev diagnostično najpomembnejša za razvoj imunske tromboze, pa njihovo delovanje še ni razjasnjeno. Do sedaj je veljalo, da so protitelesa proti beta-2-glikoproteinu I nizko avidna, najnovejši podatki pa dokazujejo obstoj tudi visoko avidnih tovrstnih protiteles. Nameravali smo pridobiti čist Fab fragment visoko avidnih protiteles proti beta-2-glikoproteinu I v nativni konformaciji, tako da bi bil sposoben vezave na antigen in bi omogočal študij patogenih mehanizmov. Protitelesa proti beta-2-glikoproteinu I smo prečistili iz ostankov materiala po terapevtsko indicirani imunoadsorpciji s pomočjo afinitetne kromatografije. Na podlagi kaotropnega ločevanja specifično vezanih protiteles na afinitetni koloni smo ločili visoko in nizko avidna protitelesa. Zaradi izredno majhnih količin izoliranih protiteles, smo postopek za cepitev protiteles z encimom papainom optimizirali za zelo razredčene raztopine z daljšimi časi cepitve in frakcijskim dodajanjem encima. Za ločevanje fragmentov protiteles smo uporabljali 10% poliakrilamidni gel v elektroforezi pod nativnimi pogoji. Po končani elektroforezi smo izvajali elektroprenos proteinov na nitrocelulozno membrano. Pri imunski detekciji na membrani smo uporabili protitelesa proti fragmentu Fab, proti lahkim in težkim verigam imunoglobulina ter proti celemu imunoglobulinu, konjugirana z alkalno fosfatazo ali peroksidazo. Z imunodetekcijo na nitrocelulozni membrani smo potrdili prisotnost iskanih fragmentov. Samo delna vezava beta-2-glikoproteina na elektroforezno prenesen fragment Fab na nitrocelulozni membrani nakazuje potrebo konformacijske spremembe molekule beta-2-glikoproteina I, ki omogoči izpostavitev skritih epitopov. Iz biološkega materiala smo uspešno izolirali visoko avidna protitelesa proti beta-2-glikoproteinu I in pridobili čisti fragment Fab, ki je zadržal svoje visoko avidne vezavne sposobnosti.

Acquisition of Fab Fragment of High Avidity Class G Immunoglobulins Against Beta-2-glycoprotein I

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Anti-beta-2-glycoprotein I antibodies is one of the main subsets associated with autoimmune disorder called antiphospholipid syndrome. Despite their clinical importance in development of immune thrombosis, their pathogenic clue has not been clarified yet. They are generally believed to be of low avidity but recent reports suggest the existence of high avidity anti-beta- 2-glycoprotein antibodies. The aim of our study was to get purified Fab fragment of high avidity anti-beta-2-glycoprotein I antibodies in native conformation, which could be able to bind the antigen in pathogenic studies. Anti-beta-2-glycoprotein antibodies were obtained by affinity chromatography from human material after therapeutic indicated immunoabsorption. High and low avidity antibodies were separated with chaotropic elution from affinity column. The digestion procedure by enzyme papain was optimised for low concentration of antibodies by prolongation of digestion and fractional addition of the enzyme. The fragments of antibodies were separated by 10% non-reducing polyacrylamide electrophoresis. Separated fragments were transferred from gel to nitrocellulose membrane. In immunodetection antibodies against Fab fragment, light and heavy chains of immunoglobulins were used, conjugated with alkaline phosphatase or peroxidase. Immunodetection on nitrocellulose membrane confirmed existence of searched fragments. Only partial

binding of beta-2-glycoprotein I to the electrophoretically transferred Fab fragments onto nitrocellulose membrane indicated the need of conformational changes of beta-2-glycoprotein I molecule for the exposure of cryptic epitope. We purified high avidity anti-beta-2-glycoprotein antibodies from human material and obtained Fab fragment of high avidity antibodies, which detained their high avidity binding properties.

Vlaganja domačega in tujega transnacionalnega podjetja v razvoj regije (primer Krke in Revoza)

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Podjetja si dolgoročen obstoj in uspeh ne zagotavljajo zgolj z ustvarjanjem dobička, temveč tudi z družbeno odgovornim poslovanjem. Velika transnacionalna podjetja, katerih poslovanje je nenehno na očeh javnosti, vlad in protiglobalizacijskih skupin pritiska, morajo svoje poslovanje uskladiti s potrebami svojih zaposlenih, dobaviteljev, potrošnikov, delničarjev in lokalnega okolja, v katerem delujejo. Dejstvo je, da so transnacionalna podjetja najbolj povezana z lokalnim okoljem v svoji matični državi. V matični državi se običajno nahajajo strateške funkcije podjetja (sedež, R&R), ki zahtevajo močno vpetost podjetja v lokalno okolje. Politika vlaganj podružnic v tujini je odvisna predvsem od zahtev sedeža, ki so bolj usmerjene na doseganje ekonomskih rezultatov podružnice kot na vlaganje podružnice v lokalno okolje. Po drugi strani pa čedalje večja potreba transnacionalnih podjetij po lokalnih dobaviteljih odpira možnost za tesnejše povezovanje tujega podjetja z okoljem, v katerem posluje.
Primerjava dveh transnacionalnih podjetij v dolenjski regiji, Krke in Revoza, je potrdila postavljeno hipotezo, da domače podjetje vlaga več v razvoj regije kot tuje podjetje. Analiza anketnega vprašalnika za zaposlene v Krki in Revozu ter za ostale prebivalce dolenjske regije je pokazala, da Revoz po mnenju anketirancev dohiteva Krko na področju vlaganj v zaposlene, lokalne dobavitelje in varovanje okolja. Največ razlik med obema podjetjema nastaja na področju sponzorstev in donatorstev, kjer Krka prispeva bistveno več sredstev za regijo kot Revoz in zato v regiji uživa tudi večji korporativni ugled.

Investing of Domestic and Foreign Transnational Company in Regional Development (Case Study of Krka and Revoz)

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Companies cannot ensure themselves long-term existence and success merely by making profit but also with socially responsible behaviour. Large transnational companies, whose performance is disclosed to public, governments and anti-globalist pressure groups, have to achieve a workable balance between their interests and the needs of their employees, suppliers, customers, shareholders and local environment in which they operate. The fact is that transnational companies are more involved in the local environment in their home country. There are usually located strategic corporate functions (headquarters, R&D) that tend to be very strongly embedded within their local milieu. Investment policy of foreign subsidiaries depends mostly on the headquarters' requirements which are directed more at achieving good economic performance than investing and supporting local community. On the other hand the growing need of transnational companies for local suppliers has opened up opportunity for greater corporate embeddedness with environment within which company operates.
The comparison of two transnational companies in Dolenjska region, Krka and Revoz, has confirmed a hypothesis that domestic companies invest more in regional development than foreign companies. The analysis of a questionnaire among Krka and Revoz employees and other residents of Dolenjska region has shown that Revoz invests almost as much as Krka in his employees, suppliers and environment protection. Greater differences occur between both companies in the field of sponsorship and donations, where Krka contributes more to the region than Revoz and therefore also enjoys greater corporate reputation.

Gensko spremenjena hrana in varstvo potrošnikov

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Genski inženiring je gotovo najmočnejše orodje, ki ga je doslej razvil človek za preoblikovanje narave. Tovrstna tehnologija se uporablja tudi v poljedelstvu, živinoreji in živilstvu. Največji proizvajalci gensko spremenjenih poljščin so ZDA, Argentina, Kanada in Kitajska, medtem ko EU nasprotuje in zavrača tovrstne izdelke, saj njihova ureditev ščiti potrošnika, med tem ko je severnoameriška ureditev pa je zelo ohlapna. Raziskava nam je pokazala, da so na našem trgu prisotni gensko spremenjeni izdelki, le da za vse še ne vemo. Zato ima potrošnik pravico do izbire med nespremenjeno in spremenjeno hrano, saj Slovenija uvozi več hrane kot pa je sama pridela. Tako se pojavlja na naših policah tudi gensko spremenjena hrana, vendar trg mora zagotoviti potrošnikom najvišjo stopnjo blaginje ter možnost izbire. Tu pa se pojavi ekološko kmetovanje, ki je pravo nasprotje tovrstni hrani, zato je njegovo glavno vodilo gospodarjenje v skladu z naravo, saj tovrstni proizvodi ne vsebujejo kakršnih koli snovi, ki kvarno vplivajo na naše zdravje. Potrošniki, zavedajte se, da imate možnost izbire, samo uveljaviti morate svojo voljo.

Genetically Modified Food and Customer Safety

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Genetic engineering is definitely the most powerful weapon humans made to alter nature. Technology of this kind is used in agriculture and food industry. The biggest producers of genetically modified crops are USA, Argentina, Canada and China. EU does not approve and rejects products of this kind and its policy protects the customer much more than the North-American. The research has shown that our markets offer genetically modified products yet we do not recognize all of them. This is why customers have the right to choose between altered and unaltered food. In Slovenia, more food is imported than produced. And because of the fact that we are offered also genetically modified food, the market should provide the highest level of welfare and the option of choice. For example ecological farming. It is the opposite of altered food industry with its main principle to use only nature-compatible products, which do not affect our health. Customers must realize they have a choice. They just have to assert their rights.

Uporaba statističnih metod in umetnih nevronskih mrež v retrospektivni analizi procesa granuliranja

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Z uporabo metode glavnih komponent, multiple linearne regresije in umetnih nevronskih mrež smo razvili model za optimizacijo procesnih parametrov pri vlažnem granuliranju, z namenom, da izboljšamo sproščanje učinkovine iz kapsul, oziroma zmanjšamo variabilnost rezultatov sproščanja.
V dveh podatkovnih bazah, v katerih so bili zbrani procesni parametri za 100 mg in 200 mg kapsule, smo najprej z metodo glavnih komponent in multiplo linearno regresijo razvili model za napovedovanje sproščanja na podlagi danih podatkov. S pomočjo navzkrižnih vrednotenj smo določili optimalne parametre učenja nevronskih mrež, ovrednotili napovedno moč modelov in izbrali najboljši model za napovedovanje. Ugotovili smo, da z umetnimi nevronskimi mrežami boljše napovedujemo sproščanje. Poskus na 200 mg kapsulah v proizvodnem merilu je pokazal, da je naš model dober, saj je bila največja napaka napovedi sproščanja z umetno nevronsko mrežo 7%, z multiplo linearno regresijo pa 12%.

Application of Statistics and Artificial Neural Networks in Retrospective Analysis of a Granulation Process

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By using the principal components analysis, multiple linear regression and artificial neuron nets, we developed a model for optimizing the process parameters in granulation process in order to improve the release of the active ingridient from the capsules and/or decrease the variability of the release results.
We gathered the process parameters for 100 mg and 200 mg capsules in two databases. First we developed a model for predicting release based on the given data by using the principal components analysis and multiple linear regression. With the halp of cross-evaluations, we determined the optimal learning parameters of the neuron nets, assessed the prediction strenght of the models and selected the best prediction model. We discovered that by using artificial neuron nets, the release prediction is more accurate. The experiment with 200 mg capsules on production size sample, showed that our model was good, as the biggest mistake of the release prediction by using an artificial neuron net was 7% and 12% when using multiple linear regression.

Gen za klitocipin, inhibitor cisteinskih proteaz, pri izbranih vrstah gliv

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Klitocipin je nov inhibitor cisteinskih proteaz, izoliran iz glive *Clitocybe nebularis*. Od ostalih inhibitorjev cisteinskih proteaz se razlikuje v aminokislinskem zaporedju in spektru inhibicije, kar ga uvršča v novo naddružino mikocipinov. Prisotnost genskega zapisa za strukturni del, promotorsko in terminatorsko regijo je bila ugotovljena pri več vrstah bazidiomicet, pri nekaterih so izolirali tudi protein. Z uporabo metode PCR smo določili nukleotidno zaporedje strukturnega dela gena za klitocipin pri glivah *Clitocybe geotropa*, *Clitocybe alexandri*, *Lepista nuda* in *Armillaria mellea*, ki je dolgo 670 bp (oziroma 681 bp pri glivi *Armillaria mellea*) in vsebuje tri introne, v velikosti približno 60 bp. Analiza nukleotidnih zaporedij je pokazala možnost obstoja povezave med filogenijo gob in podobnostjo v genu za klitocipin. Nadalje smo določili delno nukleotidno zaporedje strukturnega dela gena za klitocipin pri glivah *Macrolepiota procera*, *Amanita lividopallescens*, *Suillus granulatus*, *Lactarius citriolens* in *Helvella crispa*. Kaže, da v vseh proučevanih glivah, z izjemo gliv *Amanita lividopallescens* in *Helvella crispa*, obstajata dve različici nukleotidnega zapisa za klitocipin, od katerih ena vsebuje prvi intron, druga pa ne. Določena delna zaporedja fragmentov brez introna se med vrstami in celo med debloma bazidiomicet in askomicet ne razlikujejo in vsebujejo iste mutacije v primerjavi z nukleotidnimi zaporedji, ki imajo prvi intron. Možno je, da v celicah gliv obstaja več različnih tipov klitocipina, ki so posledica značilnih razlik v nukleotidnem zaporedju njihovih genov in imajo različne fiziološke vloge.

Gene for Clitocypin, Cistein Protease Inhibitor, in Selected Species of Fungi

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Clitocypin is a novel cysteine proteinase inhibitor, isolated from fungus *Clitocybe nebularis*. Its amino acid sequence and inhibition spectra differ from those of other cysteine protease inhibitors. That classifies clitocypin as a member of a new super family, micocypines. The presence of the structural gene, promoter and terminator region was confirmed in some of the basidiomycetes. The protein was isolated from some of them. We determined the nucleotide sequence of the structural gene for clitocypin in fungi Clitocybe geotropa, *Clitocybe alexandri*, *Lepista nuda* and *Armillaria mellea*, using PCR. The sequence consists of 670 bp (681 bp in fungus *Armillaria mellea*) and contains three introns 60 bp in size. Nucleotide sequence analysis showed there might be a correlation between phylogeny of fungi and similarity of the genes. Partial nucleotide sequence was determined in fungi *Macrolepiota procera*, *Amanita lividopallescens*, *Suillus granulatus*, *Lactarius citriolens* and *Helvella crispa*. It seems that in fungi, except in *Amanita lividopallescens* and *Helvella crispa*, two versions of the gene for clitocypin exist, namely the one containing the first intron and the other without it. The latter does not differ between species (even between *basidiomycota* and *ascomycota*) and has same mutations in comparison with the former. It is possible that more types of clitocypin exist in the cells, which have characteristic differences in the nucleotide sequence and specific physiological roles.

Termodinamska stabilnost proteina MazE, ki sodeluje pri programirani celični smrti bakterij

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Toplotna denaturacija MazE je bila študirana s pomočjo diferenčne dinamične kalorimetrije (DSC), spektropolarimetrije (CD) in fluorescenčne spektroskopije v pH območju med 3,1 in 9,4. Denaturacijo MazE pri vseh merjenih pH spremlja povečevanje CD signala v daljnjem UV območju. Ta prvič opažena značilnost pri proteinih je zelo verjetno posledica prevlade prispevka disociacije MazE-dimera na nad razvijanjem polipeptidne verige. V pH območju med 3,1 in 4,5 pri T < 30 °C MazE obstaja v "molten globuli" podobnemu stanju z delno porušeno terciarno strukturo in ohranjeno sekundarno strukturo. Modelska analiza DSC in CD "talilnih" krivulj pokaže, da je toplotna denaturacija MazE reverzibilen enostopenjski prehod med nativnim MazE-dimerom in denaturiranima monomeroma pri pH > 3,1. Pri nizkih koncentracijah MazE je lahko tudi pri fizioloških pogojih (37 °C in pH ~ 7) prisotno zaznavno število molekul MazE v denaturiranem stanju. MazE je najbolj stabilen pri fiziološkem pH ~ 7. Njegova stabilnost pada z naraščanjem temperature v območju 3,1 < pH < 9,4. Ker je stabilnost MazE relativno nizka, je presenetljivo, da MazE ohrani del sekundarne structure celo pri 100 °C. To pomeni, da del MazE (hidrofobno jedro) ostane strukturiran, čeprav v proteinu ni prisotnih disulfidnih vezi.

Thermodynamic Stability of the Bacterial Protein MazE That Takes an Active Part in the Programmed Cell Death of Bacteria

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The thermal denaturation of MazE was studied using differential scanning calorimetry (DSC), spectropolarimetry (CD) and fluorescence spectroscopy in a pH range between 3,1 and 9,4. The denaturation of MazE in the entire pH range is accompanied by significant increase of CD signal in a far UV range. This unique feature is most probably a consequence of domination of MazE-dimer dissociacion over the unfolding of its polypeptide chain. In the pH range between 3,1 and 4,5 at T < 30 °C MazE exists in a "molten globule" like state with a partially destroyed tertiary structure and preserved secondary structure.
Model analyses of CD and DSC melting curves show that the denaturation of MazE is a two state reversible dimmer-monomer transition at pH > 3,1. At lower concentrations of MazE its denaturated state is significantly populated even at physiological conditions (37 °C, pH ~ 7). MazE shows the greatest stability at physiological pH ~ 7. Its stability decreases with increasing temperature in the 3,1 < pH < 9,4 range. Since MazE has relatively low thermodynamic stability it is surprising that both secondary and tertiary structure are not completely lost even at 100 °C, meaning that a part of MazE (hydrophobic core) remains structurated in the monomeric form despite no presence of disulphide bonds in the protein.

Razvoj hkratne analize *Bsm*I in *Fok*I polimorfizmov v genu za receptor vitamina D

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Že leta 1994 so dokazali, da je mineralna gostota kosti (BMD, angl. *Bone Mineral Density*) povezana s spremembami (polimorfizmi) gena za receptor vitamina D (VDR). Če bi dokazali povezanost kombinacije (haplotipa) polimorfizmov z nizko BMD, bi dobili bolj zanesljiv kazalec tveganja za razvoj osteoporoze. Dobili bi možnost odkrivanja bolezni mnogo pred kliničnimi znaki in nova izhodišča za načrtovanje preprečevanja in zdravljenja te bolezni.
Da bi skrajšali čas analize ter zmanjšali porabo reagentov pri proučevanju dveh pomembnejših polimorfizmov v genu za VDR, smo na novo razvili metodo hkratne analize *Fok*I in *Bsm*I polimorfizmov. V študijo smo vključili 83 bolnikov, ki jim je bila opravljena artroplastika. Vzrok za operacijo je bila osteoporoza ali osteoartroza.
Poiskali smo optimalne pogoje za hkratno verižno reakcijo s polimerazo (angl. *Multiplex PCR*), da smo v isti reakciji pomnožili dva ločena odseka gena za VDR, in sicer 266 bp eksona 2 in 461 bp introna 8. Nadalje smo poiskali dovolj selektivne pogoje za analizo polimorfizmov dolžin restrikcijskih fragmentov (RFLP, angl. *Restriction Fragment Lenght Polymorphism*). S FokI in BsmI restrikcijskima endonukleazama smo hkrati ugotavljali polimorfizma 12022 T→C in 45082 G→A ter vsakemu preiskovancu določili haplotip.
S Post Hoc testom smo potrdili, da je haplotip BbFF statistično značilno povezan z znižano vrednostjo BMD kolka in ledvenih vretenc pri naših preiskovancih.
Razvita metoda hkratne genotipizacije *Fok*I in *Bsm*I polimorfizmov bo razpolovila čas in bistveno zmanjšala stroške omenjenih genskih analiz.

Development of Multiplex Analyse for *Bsm*I and *FokI* Polymorphisms in Vitamin D Receptor Gene

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As early as in 1994 has been reported that bone mineral density (BMD) is associated with polymorphisms of the vitamin D receptor (VDR) gene. If we determined the association of haplotypes of polymorphisms with lower BMD, we will obtained more reliable diagnostic tool to identify individuals with risk of developing osteoporosis. We will have chance to detect the disease much earlier before clinical symptoms appear, and a new accession to prevention and treatment of osteoporosis.
To reduce time and the consumption of reagents and consequently the costs of analysis we have simultaneous developed the multiplex method for analysis *Bsm*I and *Fok*I polymorphisms in the VDR gene.
83 arthroplastic patients with osteoporosis or osteoarthrosis were enrolled to the study. We tryed to find the optimal reaction conditions for Multiplex polymerase chain reaction (PCR) to amplify in the same reaction two separated fragments 461 bp in intron 8 and 266 bp in exon 2 of VDR gene. To determine haplotypes of *Bsm*I and *Fok*I polymorphisms we have to find selective reaction conditions of Restriction fragment length polymorphism (RFLP). Polymorphism 45082 G→A and 12022 T→C were detected by using *Bsm*I and *Fok*I restriction endonucleases.
After multiple comparissons (Post Hoc tests) we determine that haplotype BbFF is associated with significantly lower hip and lumbar spine BMD in our arthroplastic patients.

Sinteza indolskih analogov RGD peptida

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Tripeptidna RGD-sekvenca (Arg-Gly-Asp) je celično prepoznavni motiv odgovoren za vezavo ligandov, kot so fibronektin, fibrinogen, vitronektin, laminin, von Willebrandov faktor in osteopontin na različne podtipe integrinskih receptorjev.
Z integrinskimi antagonisti, RGD-peptidomimetiki, lahko preprečimo vezavo endogenih ligandov na integrine in vplivamo na potek bolezni kot so kardiovaskularna obolenja, rak, osteoporoza, ateroskleroza, revmatoidni artritis, nekontrolirana angiogeneza, diabetična retinopatija in infekcijska stanja.
Sintetizirali smo več analogov RGD-peptida, vsem pa je kot mimetik arginina skupen 5-amidinoindolski strukturni element.
Amidinsko funkcionalno skupino smo pripravili s Pinnerjevo sintezo iz ciano skupine vezane na indolski obroč. Kot mimetik C-terminalnega dela asparaginske kisline smo uvedli karboksilno skupino kot sestavni del različnih 3,5-disubstituiranih 1,2,4-oksadiazolov, oziroma kot del D- ali L- fenilalanina. Med amidinsko in karboksilno skupino smo zagotovili ustrezne razdalje, potrebne za antagonistično delovanje.
Sintetizirane spojine predstavljajo potencialne antagoniste fibrinogenskega in vitronektinskega receptorja in so lahko dobra usmeritev za nadaljna raziskovanja na tem področju.

Synthesis of Indol RGD Analouges

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The tripeptide sequence RGD (Arg-Gly-Asp) is a common cell-recognition motif, which is a part of integrin binding ligands, like fibronectin, fibrinogen, vitronectin, laminin, von Willebrand factor and osteopontin, and is responsible for their binding to different subtypes of integrin receptors.
Appropriate integrin antagonists, RGD peptidomimetics, are able to prevent the binding of endogenous ligands to integrins and thus have an important role in different diseases such as cardiovascular diseases, cancer, osteoporosis, atherosclerosis, rheumatoid disorders, uncontrolled angiogenesis, diabetic retinopathy and infection renders.
We synthesized some RGD peptides all of them with 5-amidinoindole as arginine mimetic. Amidine group was prepared by Pinner synthesis from ciano group bound to indol. As a mimetic of C-terminal part of aspartic acid, carboxyl group was introduced as a part of different 3,5-disubstituted 1,2,4-oxadiazoles or by D- or L-phenylalanine.
To assure antagonistic activity, appropriate distance between amidine and carboxyl group was determined.
Prepared molecules are potential fibrinogen and vitronectin receptor antagonists and could be a good direction for further resarch work in this field.

Izolacija in karakterizacija rekombinantne človeške 17β-hidroksisteroid-dehidrogenaze tip 5

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17β-hidroksisteroid-dehidrogenaza tip 5 (17β-HSD tip 5) je hidroksisteroid-dehidrogenaza, ki spada v proteinsko naddružino aldo/keto reduktaz. Hidroksisteroid-dehidrogenaze so pomembne pri biosintezi in uravnavanju aktivnosti estrogenov, androgenov in progestagenov ter so lahko povezane z nastankom hormonsko odvisnih oblik raka. V okviru diplomske naloge smo izbrali optimalne pogoje pridobivanja 17β-HSD tip 5 v obliki fuzijskega proteina z glutation-S-transferazo v *E.coli*. Rekombinantni encim smo očistili v eni stopnji z afinitetno kromatografijo. Določili smo substratno specifičnost, kinetične parametre za progesteron, androstendion in za nefiziološki substrat 9,10-fenantrenkinon, temperaturno stabilnost encima in ugotavljali izražanje gena *17ß-HSD tip 5* pri raku endometrija. Ugotovili smo, da rekombinantni encim deluje kot NAD(P)/H odvisna 3α-, 17β-, 20α-hidroksisteroid-dehidrogenaza. Encimu se aktivnost bistveno ne spremeni, če ga inkubiramo pri 2 °C, pri daljši inkubaciji pri sobni temperaturi pa aktivnosti ni več zaznati. Študije izražanja gena *17ß-HSD tip 5* v vzorcih rakastega tkiva endometrija z metodama RT PCR in PCR v realnem času so pokazale, da je izražanje gena povečano v 4 oz. 6 vzorcih raka endometrija, v 3 je izražanje zmanjšano, pri enem pa ni razlike. Čeprav bo potrebno študije izražanja izvesti na večjem številu vzorcev, pa bosta izolacija in karakterizacija rekombinantne 17β-HSD tip 5 omogočili nadaljnje preučevanje potencialnih inhibitorjev, ki bi se morda lahko uporabili kot zdravilne učinkovine pri zdravljenju raka endometrija.

Isolation and Characterization of Recombinant Human 17β-hydroxysteroid-dehydrogenase Type 5

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17β-hydroxysteroid-dehydrogenase type 5 (17β-HSD type 5) is a hydroxysteroid-dehydrogenase that belongs to the aldo-keto reductase protein superfamily. Hydroxysteroid-dehydrogenases are involved in biosynthesis and the regulation of estrogen, androgen and progestagen action and therefore play an important role in the development of hormone dependent forms of cancer. In our study, we expressed 17β-HSD type 5 as fusion protein with Glutathione-S-Transferase in *E.coli* and purified the recombinant enzyme by affinity chromatography. We tested a variety of induction parameters to provide a greater yield of fusion protein in the soluble fraction. We determined the kinetic parameters for progesterone, androstenedione and for nonphysiological 9,10-phenatrenequinone, studied substrate specificity and thermal stability. We also investigated expression of *17ß-HSD* type 5 in samples of human endometrial cancer. Recombinant 17β-HSD type 5 acted as a NAD(P)/H-dependent 3α-, 17β-, 20α-hydroxysteroid-dehydrogenase. Enzyme activity did not change much when incubated at 2 °C while after prolonged incubation at room temperature enzyme activity was not detected. The RT PCR and Real time PCR studies revealed higher *17ß-HSD type 5* expression in 4 or 6 samples of endometrial cancer, lower in 3 and the same in one sample. Although *17ß-HSD type 5* expression should be examined in larger number of samples, the isolation and characterization of 17β-HSD type 5 enables further inhibition studies that might lead to the development of new drugs for treatment of endometrial cancer.

Novi inhibitorji antigena 85C

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Tuberkuloza (TBC), infekcijska bolezen, predstavlja v današnjem času zaradi pojavljanja rezistentnih sevov glavni vzrok smrti, povzročene s strani enega samega patogena. Med novejšimi tarčami za razvoj novih učinkovin je v zadnjem času pritegnil pozornost kompleks antigen 85. To so ekstracelularni proteini povzročitelja TBC *Mycobacterium tuberculosis*, sestavljeni iz treh sorodnih sestavnih delov (Ag85A, Ag85B, Ag85C). Kompleks Ag 85 je poleg vezave fibronektina in antigenskih lastnosti sposoben tudi reakcije preestrenja (mikoliltransferazna aktivnost), pri kateri iz dveh molekul trehaloze monomikolata nastane trehaloza dimikolat. Trehaloza dimikolat (imenovan tudi »cord factor«) je za obstojnost zunanjega lipidnega dvosloja stene mikobakterij (in posledično za njihovo preživetje) ključnega pomena. Na osnovi poznavanja 3D strukture Ag85C in prepostavljenega mehanizma katalize ter s pomočjo molekulskega modeliranja smo načrtovali in sintetizirali nove reverzibilne inhibitorje tega encima. Kot mimetike trehaloze smo pri tem uporabili nekatere enostavne alkohole, kot npr.: 3-fenoksibenzilni alkohol, N-(hidroksimetil)ftalimid. Za mimetike mikolne kisline pa smo izbrali različne alkilne verige (od 2 do 4 C atomov dolžine), ki so na koncu substituirane s ftalimidnim oz. benzenovim aromatskim obročem. Oba fragmenta smo povezali z etil fosfonatno funkcionalno skupino in na ta način dobili serijo poenostavljenih analogov tetraedričnega prehodnega stanja.

New Inhibitors of Antigen 85C

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Tuberculosis (TB), an infection, is nowadays the leading cause of death from a single infectous agent due to the emergence of drug-resistant strains.Consequently, new approaches to the treatment of TB are needed. The antigen 85 (Ag85) complex, is a major protein component of the cell wall of *Mycobacterium tuberculosis*, the causative agent of TB. Ag85 is composed of three proteins (Ag85A, Ag85B and Ag85C). Beside the antigenic properties and fibronectin binding capabilities, the complex possesses a mycolyltransferase activity, where trehalose 6,6'-dimycolate is formed by catalyzing transfer of mycolic acid from one trehalose 6-monomycolate to another, all greatly contributing to cell wall biosynthesis. Disruption of the TDM forming by inhibition of Ag85C mycolyl transferase activity is thus a promising strategy for the development of novel anti-TB agents. Crystal structure of Ag85C and its proposed catalytic mechanism allowed us to use computer-aided structure-based design of new reversible inhibitors of the Ag85C mycolyltransferase activity. We use simple alcohols like 3-phenoxybenzyl alcohol, benzyl alcohol and N-(hydroxymethyl)phtalimide to mimic the trehalose. Phtalimide and benzene substituated alkyl chains (C-2 to C-4) were used to mimic the mycolic acid. The two fragments were linked by an ethyl phosphonate moiety to give a simplified tetrahedral transition-state analogues.

Študij interakcij transkripcijskih faktorjev družin SREBP in CREM/CREB ter vpliv fosforilacije pri uravnavanju prepisovanja človeškega gena *CYP51*

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Ugotovili smo, da imajo različni predstavniki družine transkripcijskih faktorjev SREBP v prisotnosti PKA različno sposobnost za transaktivacijo človeškega gena *CYP51*. Zanimivo je, da se je izmed vseh izooblik SREBP za najmočnejšega izkazal SREBP-2gc. V naših poskusih fosforilacija s PKA ni vplivala na CREB- in s CREMτ-posredovano prepisovanje. Pri študiju interakcij med transkripcijskimi faktorji SREBP-1a in CREB/CREMτ smo ugotovili, da CREB in CREMτ pri visokih koncentracijah tekmujeta s SREBP-1a za vezavo na promotor CYP51. Vezavni mesti za CREB/CREMτ (CRE2) in SREBP-1a (SRE1) na promotorju CYP51 sta namreč ločeni samo za 11 bp, zato pri visokih koncentracijah transkripcijskih faktorjev pride do nasičenja vezavnih mest. Predpostavljamo, da prevlada tisti transkripcijski faktor, ki ima večjo afiniteto do svojega vezavnega mesta. V tem primeru sta to CREB in CREMτ. Pri tem je CREB sposoben v celoti izpodriniti SREBP-1a s promotorja CYP51, CREMτ pa le delno, kar je lahko posledica manjše afinitete do vezavnega mesta CRE2 v primerjavi s CREB.

Study of Interactions Between Transcription Factors of the SREBP and CREM/CREB Families and the Phosphorilation Effect in Regulation of the Human *CYP51* Gene Expression

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We have established that different transcription factors of SREBP family have different ability to transactivate human *CYP51* gene in the presence of PKA. Very interesting is the fact, that SREBP-2gc has shown to be the most potent among all SREBP isoforms. In our experiments PKA did not have any influence on the CREB- and CREMτ-mediated transcription of human CYP51 gene. In our studies of interactions between transcription factors SREBP-1a and CREB/CREM τ we have established, that in high concentrations CREB and CREMτ are competing with SREBP-1a for binding on CYP51 promoter. The most probable reason for this phenomenon is in the fact that the binding sites for CREB/CREMτ (CRE2) and SREBP-1a (SRE1) are only 11 bp apart. Therefore at high concentrations of transcription factors the binding sites become saturated. We presume that the transcription factorm, whose affinity to it´s binding site is higher, wins. In this case the winners are CREB and CREMτ. We have realised that CREB can totally supersede SREBP-1a from the CYP51 promoter and CREMτ only partially, which could be the result of it´s minor affinity to the binding site CRE2 in comparison to CREB.

Programska rešitev za mobilno podporo spremljanju zdravstvenega stanja pacienta ob bolniški postelji

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Diplomsko delo predstavlja raziskavo obstoječega sistema, z namenom da bi se podprlo tudi aktivnosti zdravljenja, opravljene ob bolniški postelji. Današnji togi bolnišnični informacijski sistemi ne podpirajo dinamičnega dela medicinskega osebja in pridobljene informacije ob bolniški postelji zato niso tako izkoriščene kot bi lahko bile. Na podlagi opravljenih analiz so bile postavljene funkcionalne zahteve in sestavljena vsebina uporabniških vmesnikov. V sklopu pomanjkljivosti spremljanja opravljenega dela je poudarek na nedoslednem spremljanju porabe zdravil. S programsko rešitvijo bodo dostopni podatki o bolnikovi terapiji ob postelji, beleženje opravljene terapije pa postavlja osnovo za sprotno spremljanje porabe zdravil, ter v prihodnosti ekspertni sistem za podporo pri predpisovanju terapije. Raziskava trga mobilnih naprav je skupaj s postavljenimi zahtevami pokazala potrebo po uporabi ne ene, ampak dveh vrst mobilnih naprav, pocket PC in tablet PC. Na podlagi analize stanja so bile narejene potrebne dopolnitve baze podatkov.

Software Solution for Mobile Support of Monitoring Patient's Healtcare at Bedside

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This work presents the research done to extend existing systems with services supporting the medical personnel conducting bedside care. Today's rigid hospital information systems do not sustain a dynamic, field work of medical personnel and information gathered at bedside are still poorly exploited. On the basis of analysis done functional requirements were set up and the content of user interface was defined. Major deficiencies are shown in inconsistence of monitoring work done, especially in inconsequent supervising of using medicaments. Software solution will make possible for doctors and nurses to access data about patient's therapy and to record varieties of activities prescribed to patient performed. Recording of drug consumption is also setting up the basis for monitoring expenditure of medicament on-line and expert system to support therapy prescribing. Market research was conducted to find devices that fulfil all design requirements. Analysis have displayed the need for two different mobile devices, pocket PC and tablet PC. On the basis of analysis of existing database some necessary supplements and adaptations were made.

Analiza polimorfizma gena za metionin sintazo in vpliva na tveganje za nastanek raka debelega črevesa pri Slovencih

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Encim metionin sintaza (MS) katalizira prenos metilne skupine pri sintezi metionina. Polimorfizem A2756G v genu za MS se odraža v zmanjšani aktivnosti MS. Ker je iz epidemioloških študij znano, da je zmanjšan vnos folata in metionina s hrano dejavnik tveganja za nastanek raka širokega črevesa, smo v naši študiji preverili domnevo, da polimorfizem MS A2756G predstavlja enega od dejavnikov tveganja za nastanek raka širokega črevesa. S pomnoževanjem dela genomske DNK z verižno reakcijo s polimerazo in cepljenjem pomnoženega odseka z encimom *Hae* III smo določili genotip MS 118 vzorcem DNK izoliranim iz tumorjev bolnikov z rakom širokega črevesa in 133 vzorcem DNK zdravih, nesorodnih oseb, ki so predstavljale slovensko populacijo. Ugotovili smo, da je razlika v pogostnosti polimorfnih alelov med obema skupinama preiskovancev statistično značilna ($p<0.05$). Tveganje za nastanek raka širokega črevesa je pri osebah z genotipom AG ali GG 1,8 krat večje (95% CI: 1,049-3,034), pri bolnikih s sporadično obliko raka pa kar 2,0 krat večje (OR = 2,021, 95% CI: 1,135-3,599) kot pri osebah z genotipom AA. Pri bolnikih s sporadično obliko raka smo ugotovili povezavo med pogostnostjo genotipov in lokacijo, stopnjo diferenciacije ali razširjenostjo tumorja. Odkritje povezave med polimorfizmom gena MS A2756G in povečanim tveganjem za nastanek raka širokega črevesa odpira nekatere nove možnosti za preprečevanje in zdravljenje te vrste raka. Pri ljudeh, ki so nosilci polimorfnega alela MS 2756G bi z večjim vnosom metionina in folata s hrano lahko zmanjšali tveganje za nastanek raka širokega črevesa. Pri tistih bolnikih z rakom širokega črevesa, ki so nosilci alela MS 2756G, pa bi uspešnost kemoterapije s 5-fluorouracilom morda lahko povečali z zmanjšanim vnosom metionina in dodatkom rekombinantnega encima metioninaze.

Analysis of the Methionine Synthase Gene Polymorphism and Susceptibility to Colorectal Cancer in Slovenian Population

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Methionine synthase (MS) catalyzes the transfer of methyl group for methionine synthesis. The A2756G polymorphism in the MS gene results in decreased MS activity. As epidemiological studies indicated that decreased dietary intake of folate and methionine predisposes to colorectal cancer (CRC) we designed a study to investigate if MS A2756G polymorphism represents a risk factor for CRC. Genotyping based on polymerase chain reaction (PCR) followed by *Hae* III digestion was used to determine the frequencies of polymorphic MS genotypes in genomic DNA samples from 118 CRC cases and 133 healthy controls representing Slovenian population. We observed a significant difference in the distribution of polymorphic MS allele frequencies between CRC patients and healthy controls ($p<0.05$). Individuals with 2756 AG or GG genotype had 1,8-fold (95% CI: 1,049-3,034) increase in the risk for CRC in comparison to individuals with AA genotype. Polymorphic MS genotype conferred even greater risk for sporadic CRC (OR= 2,021, 95% CI: 1,135-3,599), but did not represent a risk factor for familial CRC. In the group of patients with sporadic CRC an association was observed between MS genotype and location, degree of differentiation or dissemination of tumor. The finding of association between MS A2756G polymorphism and CRC risk opens new possibilities for CRC prevention and treatment. In carriers of MS 2756G allele CRC risk could be reduced by increased methionine and folate dietary intake. On the contratry in carriers of MS 2756G allele treated for CRC with 5-fluorouracil the chemotherapeutic effect could be potentiated with methionine restriction or with the addition of recombinant methioninase.

Karakterizacija gliv *Penicillium chrysogenum*, "*P. arcticum*" in "*P. svalbardense*", izoliranih iz ledeniškega ledu na Arktiki

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Leta 2001 in 2003 so v ledeniškem ledu na Arktiki (Spitzbergi) odkrili ekstremofilne glive. Prevladovale so askomicetne in bazidiomicetne kvasovke, melanizirane glive rodu *Cladosporium* in *Aureobasidium* ter različne vrste rodu *Penicillium*.
Ena od prevladujočih vrst med izolati rodu *Penicillium* je bila kozmopolitska vrsta *P. chrysogenum*. V prvem delu naloge smo želeli z molekularno karakterizacijo (predvsem AFLP) opisati razlike v in med sevi populacije vrste *P. chrysogenum*, izolirane v Arktiki z izolati iz "običajnih" okolij. Na ta način smo želeli ugotoviti ali je polimorfizem populacije *P. chrysogenum* v tesni soodvisnosti z okoljem v katerem ta populacija živi.
Taksonomske analize rodu *Penicillium* so pokazale, da so med izolati najverjetneje prisotne tudi nove vrste. Dve sta bili preliminarno poimenovani kot "*Penicillium svalbardense*" in "*P. arcticum*". V drugem delu smo karakterizirali seve obeh novih vrst, izolirane iz ledeniškega ledu in iz ostalih arktičnih vzorcev (morski led, morska voda), ki so se razlikovali od sedaj opisanih sorodnih vrst na nivoju morfologije, fiziologije in profila sekundarnih metabolitov. Spremljali smo njihovo populacijsko dinamiko glede na fizikalno–kemijske parametre in ugotavljali biokemijske ter morfološke karakteristike. Slednje so zajemale primerjavo s taksonomskimi opisi iz literature za znane vrste (*P. chrysogenum*, *P. brevicompactum*) oz. opis teh karakteristik za nove vrste.

Characterization of Fungi *Penicillium chrysogenum*, "*P. arcticum*" and "*P. svalbardense*" Isolated from Arctic Glacial Ice

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In 2001 and 2003 extremophilic fungi were found in glacial Arctic ice on Spitzberg islands. The dominant taxa were ascomycetous and basidiomycetous yeast, melanized fungi, mainly represented by the genera *Cladosporium* and *Aureobasidium* and different species of the genus *Penicillium*. One of the dominant isolated species was the ubiquitous *P. chrysogenum*. In the first part of the work differences within and between populations of *P. chrysogenum*, isolated from the Arctics and from normal environments were described. The main goal of molecular characterization (AFLP) was to establish the potential relation between population polymorphism and stressfulness of specific environment inhabited by the populations.
Taxonomic analyses of the genus *Penicillium* revealed several potential new species. Two of them were provisionally named "*Penicillium svalbardense*", and "*P. arcticum*". In the second part of the thesis, strains of both species, isolated from glacial ice and other Arctic environments (sea ice and sea water) and differing from so far described related species, were characterized on the level of morphology, physiology and secondary metabolite profiles. Their population dynamics in relation to physico-chemical parameters was followed and their biochemical and morphological characteristics determined. The comparison was made between known taxonomic descriptions of morphologically similar species listed in the literature (*P. chrysogenum*, *P. brevicompactum*) and the characteristics of the new species were described.

Vpliv slanosti okolja na uravnavano razgradnjo 3-hidroksi-3-metilglutaril-koencim A reduktaze pri halofilni črni kvasovki *Hortaea werneckii*

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Aktivnost encima 3-hidroksi-3-metilglutaril-CoA reduktaze (HmgR), ključnega encima biosinteze holesterola, je pri sesalcih in kvasovkah uravnavana tudi s proteolitično razgradnjo HmgR po poti ubikvitin – proteasom. Preučili smo razgradne vzorce in vlogo ubikvitinacije HmgR pri halofilni črni kvasovki *Hortaea werneckii* (HwHmgR) pri optimalnih pogojih rasti in pri hipo/hiperslanih pogojih.
Specifična aktivnost HwHmgR je odvisna od slanosti z minimumom aktivnosti pri srednji slanosti gojišča (10% in 17% NaCl) in maksimumoma pri hiposlanih (0% in 5% NaCl) in hiperslanih pogojih (25% NaCl) rasti *H. werenckii*. Z western analizo smo pokazali, da so *in vivo* proteolitični razgradni produkti HwHmgR odvisni od slanosti okolja. Dokazali smo, da se HwHmgR zagotovo ubikvitinira, vendar se način ubikvitinacije razlikuje glede na slanost okolja. Eden izmed izoencimov HwHmgR se nahaja v mitohondrijih, drugi pa v endoplazmatskem retikulumu. Pri mitohondrijskem izoencimu nismo opazili razlik v razgradnji pri različnih slanostih. Proteolitična razgradnja izoencima iz endoplazemskega retikuluma pa je odvisna od slanosti okolja in ustreza profilu aktivnosti encima HwHmgR. Polno aktiven dimer HwHmgR je prisoten samo pri hipo/hiper slanih pogojih, pri srednji slanosti pa je popolnoma razgrajen.
Rezultati doprinašajo k razumevanju od stresa odvisnega uravnavanja encimske aktivnosti HwHmgR pri halofilni črni kvasovki *Hortaea werenckii*.

Salt Stress Regulated Degradation of 3-Hydroxy-3-methylglutaryl-CoA Reductase in Halophilic Black Yeast *Hortaea werneckii*

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In both mammals and yeast, the activity of 3-hydroxy-3-methylglutaryl-CoA reductase (HmgR), a key enzyme of cholesterol biosynthesis, is also regulated with proteolytic degradation of HmgR by ubiquitin-proteasome pathway. We examined the degradation patterns and HwHmgR ubiquitination in the halophilick black yeast *Hortaea werneckii* under salt stress and optimal growth.
The specific activity of the HwHmgR depends on environmental salinity with significant increase at hypo/hypersaline stress conditions when compared with minimum activity at optimal growth conditions (10% and 17% of NaCl). As determined by western blot analysis the *in vivo* proteolytic degradational patterns of the HwHmgR depends on environmental salinity. Immunoblots demonstrated that ubiquitin coimunoprecipitated with HwHmgR but with significant differences in the pattern of ubiquitinated HwHmgR at different environmental salinity. We localized two isoforms of HwHmgR: one into mitochondria that shows no differences in degradation under various salinity and the other one into endoplasmic reticulum which does behave in the salt stress dependent manner that corresponds to the pattern of HwHmgR enzyme activity. Fully active HwHmgR dimer was present only under hypo/hypersaline conditions wherease at the intermediate salinity it was completely degraded.
Salt stress responsive ubiquitination of HwHmgR may contribute toward understanding the stress dependent enzyme activity.

Nova generacija monoklonskih protiteles proti rekombinantnemu humanemu prionskemu proteinu

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Ena izmed trenutno najaktualnejših potencialnih možnosti diagnostike in terapije prenosljivih spongiformnih encefalopatij temelji na uporabi monoklonskih protiteles, usmerjenih proti različnim epitopom prionskega proteina (PrP). Pripravili smo panel protiteles, usmerjenih proti rekombinantnemu humanemu PrP (recHuPrP). Z imunizacijo transgenskih *Prnp*$^{0/0}$ miši in sledečo fuzijo vraničnih limfocitov z mielomskimi celicami smo pridobili hibridome, ki proizvajajo protitelesa, usmerjena proti epitopom prisotnim na recHuPrP. Hibridomske linije, proizvajalke specifičnih protiteles, smo izbrali na podlagi rezultatov presejalnih testov, ki delujejo na osnovi metode ELISA. Najuspešnejše hibridomske linije smo nato klonirali z metodo omejenega redčenja in izbrali štiri klone, ki proizvajajo specifična, visoko afinitetna protitelesa podrazreda IgG2a. Slednja smo očistili ter jih okarakterizirali s pomočjo metode Western prenosa. Pridobljena protitelesa so, glede na rezultate analiz, usmerjena proti različnim epitopom molekule humanega PrP. Z lastnim panelom protiteles lahko opazujemo heterogenost celične oblike PrP v različnih tkivih.

New Generation of Monoclonal Antibodies Against Human Recombinant Prion Protein

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Monoclonal antibodies directed against different epitopes on molecules of the prion protein (PrP) are at present, one of the most promising diagnostic and therapeutic tools for transmissible spongiform encephalopaties. A panel of mAbs directed against recombinant human PrP (recHuPrP) was prepared. Isolated spleen lymphocytes from immunised transgenic Prnp knockout mice (*Prnp*$^{0/0}$ mice) were fused with murine myeloma cells to obtain hybridoma cells. Hybridoma cell lines producing specific antibodies were screened with indirect ELISA test against recHuPrP. The selected hybridoma cell lines were then cloned by limited dilution and four specific, very potent mAbs against recHuPrP were obtained. All mAbs belonged to the IgG2a subclas. The cloned antibodies were purified and characterized by Western blot analyses. The differences in immunoblots indicated, that mAbs were directed to different epitopes on human PrP. The new panel of mAbs is a very useful tool in studying the cell form of PrP heterogeneity in different tissues.

Primerjalno vrednotenje sproščanja učinkovin različnih velikosti skozi ogrodne strukture tablet

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Celulozni etri kot hidrofilni polimeri predstavljajo ogrodje številnim tabletam s prirejenim sproščanjem. Tovrstni polimeri tvorijo ob stiku z vodo gelsko plast okoli tablete, ki omejuje raztapljanje in difuzijo učinkovine ter narekuje mehanizem nadzorovanega sproščanja.
Namen naše naloge je bil ugotoviti, kako na mehanizem in kinetiko sproščaja vpliva: razmerje med količino vgrajene zdravilne učinkovine in polimerom; velikost molekule zdravilne učinkovine in vrsta polimera. Kot modelni učinkovini smo uporabili v vodi dobro topni učinkovini različnih velikosti: pentoksifilin kot manjšo molekulo in 4 krat večji vankomicinijev klorid. Stisnili smo ogrodne tablete v treh različnih razmerjih med polimerom (HPC, HEC, HPMC) in učinkovino (polimer : učinkovina = 75:25, 50:50, 25:75) ter izvedli preskuse raztapljanja.
Ugotovili smo, da se hitrost sproščanja z večjo vsebnostjo polimera zmanjšuje in da velikost učinkovine vpliva na kinetiko sproščanja. Na mehanizem in hitrost sproščanja pa vplivajo tudi različne vrste celuloznih etrov (HEC > HPMC > HPC). Rezultate sproščanja je najbolje opisoval model Peppas-Sahlin, kjer k sproščanju prispevata tako difuzija učinkovine kot relaksacija polimernega ogrodja. Zato smo ovrednotili prispevek obeh mehanizmov za vse izdelane tablete in ugotovili, da je prispevek relaksacije največji za HPMC tablete; da narašča s količino sproščene učinkovine in ima večji vpliv na sproščanje večjega vankomicinijevega klorida.

Comparative Release Evaluation of Drug With Different Size Through the Matrix Tablets

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Cellulose ethers as hydrophilic polymers are often used as matrices for numerous modified release tablets. After contact with water these polymers form gel layer around the tablet, which represent a barrier for drug dissolution and diffusion and determine a mechanism of controlled release.
The purpose of our study was to investigate different parameters, influencing mechanism and drug release kinetics, such as: the ratio between incorporated drug and polymer, the size of drug molecule and polymer type. Two freely water-soluble molecules of different molecular size were used as model drugs: pentoxifylline as smaller and 4 times bigger vancomycin hydrochloride. Matrix tablets were compressed in three different ratios between polymer (HPC, HEC, HPMC) and drug (polymer : drug = 75:25, 50:50, 25:75) and dissolution studies were performed.
Results show, that drug release rate decreases with increasing polymer content and that molecular size influences drug release kinetics. Different types of cellulose ethers influence the drug release mechanism and kinetics, too (HEC > HPMC > HPC). The best description of drug release results was carried out by Peppas-Sahlin model, where drug diffusion and polymer relaxation contribute to the overall release. Therefore the contribution of both mechanisms was determined for all studied tablets and it was found out, that the contribution of relaxation: is the biggest for HPMC tablets; increases with the amount of released drug and influences more on the release of bigger vancomycin hydrochloride.

Preiskava mikrosatelitskih območij DNA pri raku želodca

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V nekaterih tkivih, npr. epitelnih celicah, se rakaste spremembe razvijejo pogosteje. Rak želodca je posledica večstopenjskega procesa genetskih in epigenetskih sprememb, ki vključujejo aktivacijo onkogenov in inaktivacijo zaviralnih genov. Različne histološke vrste kot tudi različne etiologije kažejo na to, da obstaja več različnih genetskih poti za dobro in za slabo diferencirano obliko te bolezni.
S preiskavo mikrosatelitske nestabilnosti (MSI) na različnih lokusih smo ugotavljali povezanost razvoja bolezni z napakami v genih, ki so odgovorni za popravilo DNA. Primerjava MSI s klinično-patološkimi znaki nam namreč lahko pomaga odkriti stopnjo razvoja rakavega obolenja in omogoči boljšo prognozo bolezni.
Posamezne odseke DNA, kjer smo pričakovali MSI, smo pomnožili z »unipleks« ali »dupleks« PCR-reakcijami. Samo analizo vzorcev pa smo naredili s pomočjo ločevanja produktov PCR s kapilarno elektroforezo, v povezavi z avtomatsko analizo s programskim paketom GENESCAN. Tako smo določili stopnjo podvojevanja in vrsto alelov, ki so povezani z napakami v popravljalnih genih in kažejo na splošno genetsko nestabilnost.
Primerjava MSI-H in klinično-patoloških kazalcev je pokazala naslednje: večina tumorjev je lokaliziranih v spodnji tretjini želodca, prevladuje intestinalni tip, ekspanzivna rast in slaba diferenciranost tumorja. Bezgavke večinoma niso prizadete. Globina infiltracije tumorja je manjša (najpogosteje sega v muskularis proprio in subserozo). Največ primerov je bilo diagnosticiranih v II. stadiju po TNM–klasifikaciji. Povezava klinično-patoloških kazalcev v skupinah MSI-L in MSS kaže na slabšo prognozo bolezni kot pri bolnikih z MSI-H.

Research of microsatellite DNA in gastric cancer

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In some tissues cancer develops more frequently, e.g. in epithelial cells. Gastric cancer is a consequence of a multi-step carcinogenic process and epigenetic changes, which include activation of oncogenes and inactivation of tumour suppressor genes. Different histological forms as well as different etiologies point out two different genetic pathways for well differentiated and for poorly differentiated gastric cancers.
By evaluating microsatellite instability (MSI) on different loci we tried to find out a linkage between development of the disease and affects in DNA mismatch repair genes. Comparison between MSI and clinicopathologic factors can help us to discover the state of the disease.
Segments of the DNA where MSI was expected, were amplified with »unipleks« or »dupleks« PCR. Analysis of the samples amplified by PCR was performed with capillary electrophoresis and linked to evaluation with the program package GENESCAN. We determined alleles that were duplicated and the degree of their duplication.
Correlation between MSI-H and clinicopathologic factors showed that most of the tumours were localised in the lower third of the stomach, associated with intestinal type, expansive growth of the tumour, and its poor differentiation. In most of the tumors no nodal invasion was observed. Tumours had lower depth of infiltration (the most common in muscularis propria and subserose). Most cases were discovered at stage II according to TNM classification. Correlation of clinicopathologic factors in MSI-L and MSS groups showed worse prognosis of the disease than that in MSI-H group.

Primerjava metod za določanje antioksidativnih aktivnosti sintetičnih in naravnih antioksidantov

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Antioksidanti, ki jih najdemo v rastlinskih ekstraktih, sadju, zelenjavi in pijači, imajo pomembno vlogo pri preprečevanju rakastih in drugih bolezni. Za določanje antioksidativnih aktivnosti antioksidantom je znanih več metod.
V tej študiji sem antioksidativno aktivnost proučevala z dvema metodama: z razgradnjo ß-karotena (BCB-metoda) in z izginjanjem barve 2,2'-difenil-1-pikrilhidrazila (DPPH-radikalska metoda).
Preiskovane komponente so bile čiste substance: BHT, kvercetin dihidrat, flavon, rutin in ekstrakt rožmarina. Absorbance vzorcev sem merila pri valovni dolžini 470 nm (BCB-metoda) in 515 nm (DPPH-radikalska metoda) takoj po njihovi pripravi in po termostatiranju pri 50 °C 60 minut oziroma pri sobni temperaturi 15 minut v temnem prostoru. Spremembo barve sem merila na UV/visible light spektrofotometru. Antioksidativno aktivnost sem preračunala kot odstotek inhibicije vzorčnih proti kontrolnim raztopinam, ki niso vsebovale preiskovanih komponent.
V analiznem postopku sem ocenila natančnost, točnost, ponovljivost in občutljivost metod. Namen dela sem dokazala z eksperimentalnim delom in tako določila faktorje oziroma eksperimentalne pogoje, ki vplivajo na meritve.
Za določitev ubežnikov, meritev, ki statistično odstopajo od ostalih, sem uporabila Grubbs-Beckov test. Rezultate sem statistično ovrednotila s F in t testom.

Comparison of Methods for Determination Synthetic and Natural Antioxidants' Antioxidant Activity

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Antioxidants are presented in plant extracts, consumable fruits, vegetables and beverages. They play an important role in carcinogenesis and other human disease states and they have received considerable attention as cancer chemo preventive agents. Many different methods are used for antioxidant activity determination.
The antioxidant activity was investigated with two different methods: the ß-carotene bleaching (BCB) test and the 2,2'-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method. Investigated compounds were pure substances as BHT, quercetin dihydrate, flavone, rutin and corresponding plant extract containing rosmarinic acid, as well.
The absorbance of the samples was measured at 470 nm (BCB-test) and at 515 nm (DPPH-method) immediately after the preparation and after keeping samples for 60 minutes in thermostat at 50 °C or in the dark for 15 minutes at room temperature. The changes in colour were measured on a UV/visible light spectrophotometer. Antioxidant activity was calculated as percent inhibition of oxidant versus control blank samples without investigated compound, which have properties of inhibiting autoxidation reactions.
The accuracy of the method, precision under repeatability and reproducibility conditions were determinated in this study. Experimental design was used to improve experimental work. It is used to decide which factors i.e. which experimental conditions have influence on the analytical measurement. Grubbs Beck test was used to find outliers, the measurements, which are statistically different from all others. The results were statistically evaluated using F and t tests.

Funkcijska analiza mutacije A92P človeškega gena *MLH1* v kvasovki *Saccharomyces cerevisiae*

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Ena izmed osnovnih celičnih funkcij je ohranitev informacije, zapisane v DNA, zato se mora celotni genom pred vsako celično delitvijo natančno podvojiti. Pri podvojevanju nastajajo napake, ki jih prepozna in popravi sistem za popravljanje neujemanja (MMR). Okvarjen MMR sistem je vzok za kopičenje številnih sprememb v kritičnih genih in razvoj rakavosti.
Dedni nepolipozni kolorektalni rak (HNPCC) je avtosomalna dominantna dedna bolezen, ki je povezana z mutacijami štirih genov, katerih proteini sodelujejo pri popravljanju neujemanja. Poznamo preko 200 mutacij, od katerih se večina pojavlja v genu *hMLH1* (človeški homolog 1 MutL). S pomočjo metode *delitto perfetto* smo kvasni gen MLH1 v kromosomu XIII zamenjali z nativnim človeškim homologom in delom med kodonoma 77 in 134 človeškega gena, ki je najbolj homologen delu kvasnega gena, ter testirali vpliv zamenjave G274C (posledica je zamenjava A92P v proteinu) na sposobnost kvasovke pri ohranjanju mikrosatelitskih GT-zaporedij. Rezultati so pokazali, da *hMLH1*, integriran v kvasni kromosom XIII, komplementira kvasni *MLH1* gen.
Uporabljena metoda je zato dobro izhodišče za nadaljnje funkcijske analize mutacij *hMLH1* gena. Ugotovili smo, da zamenjava A92P opazno zmanjša mikrosatelitsko stabilnost, zato menimo, da prolin poruši strukturo ATPazne domene in s tem prekine sodelovanje hMLH1 z drugimi MMR-proteini, ki je odvisno od ATP.

Functional Analysis of Human *MLH1* Gene Mutation A92P in *Saccharomyces cerevisiae*

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One of the basic cell functions is to obtain the genome integrity thus before every cell division the genome of the cell is replicated. Mutations that occur during replication are recognized and repaired by mismatch repair (MMR). Defect of MMR is a couse of accumulating mutations in crucial genes and carcinegenesis.
Hereditary non-polyposis colorectal cancer (HNPCC) is an autosomal dominant inherited disease associated with mutations in four genes encoding proteins involved in DNA mismatch repair (MMR). Over 200 mutations have been described and the vast majority occur in *hMLH1* (human MutL homolog 1). We are presenting the replacement of yeast gene with human ortholog and homologous fragment from codone 77 to 134 of *hMLH1*, respectively, with *delitto perfetto* method. Our results show that the *hMLH1* functions properly in yeast cells if integrated on chromosome XIII which is a great starting point for further functional analysis of *hMLH1* mutations.
The functional significance of G274C replacement resulting in A92P has been tested on microsatellite instability of poli-GT tandems in yeast. The A92P replacement increased the MSI. It is believed that proline bends the ATPase domain structure and disrupts the ATP-dependent cooperation of hMLH1 with other MMR proteins.

Biosinteza sterolov in celični cikel pri kvasovki *Saccharomyces cerevisiae* in pri človeških nesmrtnih hepatocitih

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MAS (mejozo aktivirajoča sterola) sta intermediata poznega dela biosinteze holesterola in ergosterola, ki lahko v pogojih *in vitro* sprožita nadaljevanje ustavljene mejoze mišjih jajčnih celic. Z našimi raziskavami smo želeli ugotoviti vpliv sterolov MAS na sporulacijo kvasovke *Saccharomyces cerevisiae*, procesu podobnem razvoju spolnih celic pri sesalcih. Časovni profili merjenih intermediatov so bili podobni profilu ergosterola, zato predpostavljamo, da je vloga intermediatov tekom sporulacije predvsem v njegovi sintezi. Z našo raziskavo smo želeli tudi ugotoviti, ali imajo MAS morda vlogo pri uravnavanju celičnega cikla. Naši rezultati nakazujejo, da MAS v celičnem ciklu evkariontov nimajo od biosinteze ergosterola/holesterola neodvisne vloge. Spremembe, ki jih povzroči dodatek MAS v gojišče HepG2 celic, se ne razlikujejo od s holesterolom povzročenih sprememb.Tako naši rezultati kažejo, da ima vlogo v uravnavanju celičnega cikla holesterol in ne MAS. Z našimi raziskavami smo tudi ugotovili, da je med sporulacijo kvasovke za nastanek mnogih sterolnih produktov ključno uravnavanje genov biosinteze ergosterola na ravni prepisovanja, saj se količina mRNA opazovanih genov spreminja skladno s količino ustreznih sterolnih produktov. Izjemo predstavlja gen *ERG11*, lanosterol 14α-demetilaza, katerega izražanje ni v skladu s količino FF-MAS.To nakazuje, je za gen *ERG11* ključnega pomena uravnavanje na po-transkripcijskih ravneh.

Sterol Biosynthesis and the Cell Cycle of Yeast *Saccharomyces cerevisiae* and Human Hepatoma Cells

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MAS (meiosis activating sterols) are late intermediates in the cholesterol/ergosterol biosynthetic pathway with the capacity to trigger reinitiation of oocyte meiosis *in vitro*. We examined the influence of MAS sterols on sporulation as a process very similar to meiosis of mammalian cells. The sterols have been measured at different times from the sporulation start. Similar ergosterol and measured sterol intermediates profiles suggest that in sporulation intermediates by themselves have no role distinct from ergosterol biosynthesis. The aim of this work was also to establish whether MAS have a role in regulation of the cell cycle. However, our results suggest that in the cell cycle MAS do not have roles distinct from ergosterol/cholesterol synthesis. The influence of exogenously added MAS on the cell cycle of HepG2 cells did not differ from the effect of cholesterol. Our results suggest that cholesterol itself, and not MAS, has a role in regulation of the cell cycle. Our investigation also pointed out an important role of transcriptional regulation in sterol synthesis during yeast sporulation, since in most cases sterol profiles were consistent to corresponding gene expression. The exception represents CYP51 (ERG11) which expression is not in accordance with the FF-MAS quantity throughout sporulation. These data suggest that the gene ERG11 is regulated primarily at post-transcriptional levels.

Vrednotenje podjetja na osnovi javno dostopnih podatkov – primer podjetja Krka

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Vrednotenje podjetja je temelj za določanje tržne vrednosti lastniških deležev za potrebe odločanja lastnikov o prodaji, nakupu in prevzemu podjetij. Vsako vrednotenje pomeni proces določanja vrednost. Vrednost sama po sebi ni dokončno dejstvo, je le ocena najbolj verjetne ocene v nekem trenutku, ko bosta pogajalca (kupec in prodajalec) v skladu s svojimi zanimanji našla skupni imenovalec. Vrednotenje podjetij v sodobnem konceptu kapitalskega trga obravnava ocenjevano podjetje kot vsako drugo finančno naložbo. V tržnih gospodarstvih, kamor se po letu 1989 uvršča tudi Slovenija, je vrednotenje podjetij sestavni del kapitalskih transakcij.
Naloga odgovarja na vprašanje, kako naj se za slovenski prostor veliko podjetje (družba Krka, d. d.) vrednoti, kolikšna je njegova končna ocena notranje vrednosti in za koliko ta vrednost odstopa od dejanske vrednosti na trgu vrednostnih papirjev. Cilj diplomskega dela je prikazati vrednotenje družbe Krka, d. d., na osnovi dveh sodobnih metod vrednotenja: metode diskontiranih denarnih tokov in metode primerljivih podjetij s kapitalskega trga. Naloga se konča s priporočilom za nakup delnic vrednotenega podjetja.

Company Valuation Based on Public Offered Data – Krka Case

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The company valuation is basic for establishing the shareholder value for the owners' needs when deciding on buying, selling or taking over companies. Every valuation means a process of setting up a value. The value in itself is not a final fact, but the valuation of the most probable estimate at the given time, when both parties (the buyer and the seller) can find the common denominator regarding their mutual interests. Company valuation within today's concepts of the capital market represents just another financial investment. Company valuation within market oriented economies, and Slovenia introduced it in 1989, is an integral part of capital transactions.
The paper addresses the issues of how a big Slovenian company (Krka, d. d.) can be valuated, what is the final internal value of the company and to what extent does this value differ from the actual one at the stock exchange market. The goal of final thesis is showing valuation of Krka company based on two modern methods: method of discounted cash flows and method of similar companies on stock markets. The paper concludes with the recommendation to buy shares of the company.

Iskanje potencialnih vezavnih mest za bakrove(II) ione na prionskem proteinu

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Prionske bolezni so skupina bolezni, med katerimi sta najbolj znani Creutzfeld-Jakobova bolezen pri ljudeh in »bolezen norih krav« pri govedu. Zaradi možnega prenosa bolezni na ljudi z uživanjem okužene govedine sta bili obe bolezni deležni precejšnje medijske pozornosti. Obe bolezni povzročata nenormalni strukturni izoformi (PrP^{Sc}) celičnega prionskega proteina (PrP^{C}), Normalna funkcija celičnega prionskega proteina je pri obeh vrstah neznana, čeprav je vedno več dokazov, da PrP^{C} sodeluje pri transportu bakrovih(II) ionov. Bakrovi(II) ioni se lahko na PrP^{C} vežejo tako na njegovem N-terminalnem delu - na območju oktapeptidnih ponovitev, kot tudi na C-terminalnem delu, vendar v literaturi to vezavno mesto še ni bilo opisano; podatki, ki so na voljo, pa so zmedeni. Ker je določanje strukture celičnega prionskega proteina težavno, sem za določitev vezavnih mest na C-terminalnem delu proteina uporabil molekularno modeliranje, ki ima svojo osnovo v kvantni mehaniki. Bakrov(II) ion sem postavil v bližino aminokislinskih preostankov D144, D147, D167, E152, E198, E200, H140 in H177. Rezultati izračunov so bili v primeru vezavnih mest v bližini aspartatnih preostankov popačeni oktaedri, v bližini glutamatnih preostankov so bili ligandi razporejeni planarno, izračun pri preostanku H177 pa je pokazal, da se bakrov(II) ion preferenčno veže na sosednji asparatatni preostanek. Predstavljena vezavna mesta verjetno ne sodelujejo pri vezavi bakrovih(II) ionov.

Determination of Potential Copper(II) Binding Sites on the Prion Protein

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Prion diseases are a group of fatal neurodegenerative diseases, Creutzfeld-Jakob disease in humans and "mad cow disease" in cattle being the two most known examples. Both diseases were given broad media attention, mainly because of the possible human infection due to consumption of contaminated beef meat. Both diseases are caused by an abnormal structural isoform (PrP^{Sc}) of the celular prion protein (PrP^{C}), whose normal cell is function still unknown, though mounting evidence suggests a role in copper(II) ions transport. Copper(II) ions can bind to two segments of PrP^{C}: the octarepeat region at the N-terminus and to the C-terminal portion, where the binding site has not been described yet. Avaliable data regarding this binding site are confusing. As the determination of the structure of the celular prion protein is met with difficulties, molecular modeling was used to determine the nature of some possible copper(II) binding sites. Copper(II) ion was placed in the vicinity of the following residues: D144, D147, D167, E152, E198, E200, H140 and H177. The results of the minimization have shown distorted octahedrons for binding sites around the aspartic acid residues, and planar ligand distribution for sites near the glutamic acid residues. For H177 the minimization showed that copper(II) ion preferentially binds to a nearby aspartic acid side chain and not to the histidine side chain. The evaluated binding sites probably do not represent valid copper(II) binding sites.

Ozkokotno rentgensko sipanje človeškega serumskega albumina v vodnih raztopinah natrijevega klorida

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Z metodo ozkokotnega rentgenskega sipanja (SAXS) smo preučevali strukturo in meddelčne interakcije v vodnih raztopinah človeškega serumskega albumina (HSA) ter raztopinah HSA v pufrih. Izbrani raztopini HSA v pufru smo dodajali tudi določene količine enostavne soli in opazovali njen vpliv na meddelčne interakcije. V skladu s pričakovanji izdatnost interakcij med makromolekulami narašča z naraščajočo koncentracijo HSA. Pri dani koncentraciji so te zelo odvisne od pH raztopine. Najšibkejše so pri pH vrednosti blizu izoelektrične točke, ker so tedaj makromolekule nevtralne. Njihova jakost nato narašča z naraščajočo razliko med pH raztopine in pH vrednostjo v izoelektrični točki, saj makromolekule HSA nosijo vse večji istoimenski naboj. Enostavna sol senči elektrostatske interakcije med makromolekulami, zato so te pri višji koncentraciji soli šibkejše.

Small-angle X-ray Scattering Study of Human Serum Albumin in Solutions of Sodium Chloride in Water

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Small-angle X-ray scattering (SAXS) technique has been used to study the particle structure and interparticle interactions in aqueous and in different buffer solutions of human serum albumin (HSA) at various concentrations. Further, the effects of the addition of simple electrolyte to the protein solution have been examined. As expected, the strength of interactions between the HSA macromolecules increased with increasing HSA concentration. At given HSA concentration, the interparticle correlations were strongly dependent on pH of the solution. Their importance were minimal in solutions with the pH value near the isoelectric point because HSA molecules are nearly neutral at these conditions. The stength of interactions between the HSA molecules then increased by altering the pH value away from the isoelectric point with concomitant increase of the charge of HSA molecules. Addition of salt to the solution caused the effect of screening between charged particles.

Temperaturna odvisnost električne prevodnosti razredčenih vodnih raztopin nekaterih alkalijskih cikloheksilsulfamatov

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Cikloheksilsulfaminska kislina in njene soli so umetna, nekalorična sladila, ki jih pogosto imenujemo ciklamati. Fizikalno kemijske lastnosti vodnih raztopin sladil nam nudijo nekatere možnosti za raziskovanje obnašanja molekul topljencev v vodi in s tem za ugotavljanje parametrov, ki so bistveni v procesu kemorecepcije molekul. Na osnovi raziskav lahko dobimo informacije o efektivni velikosti hidratiranih ionov in o jakosti medionskih sil v raztopinah.
Izmerili smo prevodnost razredčenih vodnih raztopin litijevega, natrijevega in kalijevega cikloheksilsulfamata v koncentracijskem območju med 0,0003 ≤ c (moldm^{-3}) ≤ 0,01 pri temperaturah med 5 in 35 ° C. Na osnovi kemijskega modela smo določili limitne vrednosti molskih prevodnosti ter konstante asociacije ionov. S pomočjo znanih vrednosti limitnih prevodnosti litijevega, natrijevega in kalijevega iona smo ocenili limitno prevodnost cikloheksilsulfamatnega aniona.
Ugotovili smo, da molska prevodnost pri neskončnem razredčenju v vseh treh primerih narašča z naraščajočo temperaturo. Vrednosti konstant asociacije so majhne in neodvisne od temperature, kar kaže na popolno ionizacijo preiskovanih soli v vodi. Hidrodinamični radij cikloheksilsulfamatnega aniona s temperaturo rahlo pada. Sklepamo lahko na zmanjšanje hidratacije pri višjih temperaturah, kar lahko vpliva na zmožnost vezanja na receptorje okusa in posledično na spremenjeno sladkost.

Temperature Dependent Conductivity of Diluted Water Solutions of some Alkali Cyclohexylsulfamates

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Cyclohexylsulfamic acid and its salts are artificial, non-caloric sweeteners, usually called cyclamates. They are soluble in water. Physical and chemical properties of water solution of sweeteners offer some possibility to determine most important parameters of chemoreception. Some information about the effective size of hydratized ions and molecular force interaction in the solutions could be obtained.
The molar conductivities of dilute solutions of lithium, sodium and potassium cyclohexylsulfamate in water were measured at temperatures from 5 to 35 °C in the concentration range between 0,0003 ≤ c (moldm^{-3}) ≤ 0,01. Analysis of experimental data, based on the chemical model of electrolyte solution, yields the temperature dependence of the limiting molar conductivities and the association constants. Using the known data of the limiting conductivities of lithium, sodium and potassium ions the limiting conductivities of the cyclohexylsulfamic ion were estimated.
According to our research the limiting molar conductivities increas with temperature in all investigated systems. The association constants are very small and almost independent of temperature, so all salts could be regarded as being completely ionized in the water solutions. A hydrodynamic radius of cyclohexylsulfamic anion is decreasing with the increasing temperature. It is suggested that the hydration of the cyclohexylsulfatmate ion is less pronounced at higher temperature what may influence the accession to and binding with receptors sites, thus influencing the taste properties.

Ali lahko z dodajanjem antocianinov v vino odpravimo ocetni cik?

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Ocetni cik (raven etanojske oz. ocetne kisline v vinu se zaradi delovanja bakterij zviša nad 1 g/L) velja za neodpravljivo vinsko bolezen. V literaturi sva zasledila, da se etanojska kislina acilira na glukozno enoto antocianina, pri katerem nastane estrska vez na 4. ogljikovem atomu glukoze (3. reaktivno mesto antocianina).
Ta podatek sva uporabila kot možnost odstranitve etanojske kisline v vinu. Najprej sva hotela preveriti, kolikšna količina etanojske kisline zreagira z antocianini, zato sva na začetku delala samo z raztopinami etanojske kisline in antocianini. Ker je estrenje ravnotežna reakcija, sva le-to izvedla pod različnimi reakcijskimi pogoji. Tako sva zmes segrevala, mešala in ji dodajala žvepleno (VI) kislino ter natrijev acetat, podatke o nezreagirani etanojski kislini pa pridobila s pomočjo titracije z NaOH.
Najin namen je bil odstraniti cik iz vina, in sicer tako, da sva v cikrjeno vino dodala antocianine. Ker pa so antocianini rdeče obarvani, je možno te dodajati le v rdeča vina, podatke o nevezani etanojski kislini pa sva pridobila s pomočjo destilacije vina.
Ker sva v vino dodala barvilo (antocianine), sva s spektrofotometrijo hotela preveriti, kako dodatek barvila vpliva na ton in intenziteto barve in za koliko se spremeni delež antocianinov (flavilijevega iona) v vinu. Tako dobljeno vino sva dala tudi na enološko analizo, kjer so organoleptično primerjali cikrjeno vino in cikrjeno vino z dodatkom antocianinov.

Is it Possible to Get Rid of Acetic Acid by Adding Antocyanins into Wine?

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Acetic acid (the level of ethyl acetic ester or acetic acid produced in wine by bacteria rises above 1 g/L) is believed to affect wine so much it cannot be cured. In literature we have come across the information that ethyl acetate and a glucose unit of antocyanin bond together to form an ester bond at the 4th carbon atom of the glucose (the 3rd reaction spot of the antocyanin).
We used this fact as a possibility of removing ethyl acetate from wine. First we decided to check the amount of ethyl acetate which reacts with antocyanins, therefore we started off only with the solutions of ethyl acetate and antocyanins. Esterification being a balanced reaction, we carried it out under different reaction conditions. The compound was heated and mixed with the addition of sulphuric (VI) acid and sodium acetate. The information about the nonreaction of ethyl acid was obtained by means of titration with NaOH.
Our goal was to remove volatile acidity from wine by putting antocyanins into volatile wine. Given the fact that antocyanins are red coloured they can only be added to red wine. The information about the nonreaction of ethyl acid could be obtained by means of distillation.
As we put a red colouring (antocyanins) to wine, we used spectrophotometric measurements to check how the addition of the colouring influences the hue and intensity of colour and how much it changes the rate of antocyanins in wine. The wine produced in this way underwent an enology analysis whereby volatile wine was compared to volatile wine with the addition of antocyanins.

Mikrobiološko določanje snažnosti v domačem okolju

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Raziskovalna naloga vsebuje mikrobiološko oceno higienskega stanja v naših domovih. Ugotavljale smo količino in določale vrste patogenih bakterij, ki se nahajajo v slovenskih gospodinjstvih na mestih, za katere vsesplošno menimo, da so najbolj oziroma najmanj čista (za primer smo vzele korito za pomivanje posode in straniščno školjko). S pomočjo mikrobiologov iz Mikrobiološkega laboratorija Zavoda za zdravstveno varstvo Novo mesto smo laboratorijsko analizirale vzorce, ki smo jih odvzele pri dvajsetih čimbolj raznolikih gospodinjstvih. Pri analizi smo ugotovile, da so naša stranišča bolj čista kot kuhinje, in da smo ljudje preveč površni pri čiščenju mest, ki dajejo zavajajoč vtis snažnosti. Izvedle smo tudi anonimno anketo. Ta je pokazala, da se vzrok nesnage v koritu za pomivanje posode ne skriva v neuporabi čistil za pomivalna korita, temveč da ga ljudje zaradi pomanjkanja časa preredko temeljito čistijo. Prav tako ne menjavajo dovolj pogosto gobic za pomivanje posode in jih ne sperejo dovolj dobro po vsaki uporabi, zato so le-te idealna gojišča za bakterije in plesni. Rezultati iz stranišč pa dokazujejo uporabo močnih dezinfekcijskih sredstev in rednejšega čiščenja, najverjetneje zaradi občutka večje nesnažnosti stranišč.

Microbiological Determination of Purity in the Home Environment

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The research paper contains a microbiological evaluation of the sanitary condition in our homes. We established the quantity and species of pathogenic bacteria in Slovene households on the spots we think are clean (or not clean)-our example were a kitchen sink and a toilet. With the help of the team of microbiologists of the Laboratory of microbiology of the Institute of Public Health in Novo mesto we analyzed the samples we took from twenty different households. We discovered that the bathrooms in average are cleaner than kitchens and that people are too superficial when cleaning the spots that look clean and can be misleading. An anonymous survey shows that the cause for the results of our tests is the lack of time for cleaning, not the irregular use of detergents. People also do not change the cloth for cleaning regularly and wash off the water. The results of toilet test show that the toilets are clean because strong detergents and regular cleaning, probably because of the feeling that the area is less clean.

Proizvodnja citronske kisline

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V tej projektni nalogi smo opazovale rast glive *Aspergillus niger* in njen vpliv na proizvodnjo citronske kisline. Na začetku smo glede na podatke iz literature domnevale, da bo baker (Cu) zaviral rast glive *Aspergillus niger* ter proizvodnjo citronske kisline, mangan (Mn) pa ju bo pospeševal. V drugem delu pa smo skušale ugotoviti optimalno količino Mn ionov, ki bi najbolje vplivala na rast glive. Domnevamo, da bo določena količina Mn ionov pospeševala rast glive, prevelika količina pa bo rast glive zavirala. Prav tako domnevamo, da bo pri določeni količini Mn ionov prišlo do večje produkcije citronske kisline kot pri ostalih količinah, vendar pa smo na koncu na podlagi poskusov v laboratoriju ugotovili, da nobena količina Mn ionov ni bistveno vplivala na zaviranje ali rast glive *Aspergillus niger*. Da bi dokazale vpliv Mn ionov, bi morale narediti dodatne poskuse in več ponovitev.

Production of Citric Acid

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In our research project we were observing the growth of fungus *Aspergillus niger* and its influence on the production of citric acid. At the begining of our work, we supposed that copper would obstruct the growth of fungus *Aspergillus niger* and citric acid production and that manganese would advance them. This assumption was recorded in secondary sources. In the other part of our research project we tried to find out optimal quantity of manganese ions, which would be the best for the growth of this fungus. We supposed that definite quantity of manganese ions would advance growth of fungus while too big quantity would obstruct it. We also supposed that definite quantity of manganese ions would influence citric acid production better than other quantities. But we found out that none of these quantities actually influenced the advancement or obstruction of the growth of fungus *Aspergillus niger*. This finding was based on our experiments. If we wanted to prove influence of manganese ions, we would have to do more experiments and repetitions.

Vpliv pH-ja na sestavo brinovega olja

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Natančneje smo raziskovale področje eteričnih olj. Osredotočile smo se na brinovo eterično olje.
S parno destilacijo smo pridobile kvaliteto, podobno običajni kvaliteti na trgu, ki vsebuje preveč sabinena in α-pinena, ki negativno vplivata na človekovo zdravje (prevelike količine α-pinena škodujejo nosečnicam in ledvičnim bolnikom). V literaturi smo našle podatek, da so količine določenih spojin v brinovem eteričnem olju tekom destilacije odvisne od pH vrednosti topila.
Najprej smo izračunale potrebne volumne koncentriranih kislin za pripravo raztopin klorovodikove kisline (pH=0,5, pH=1, pH=2, pH=3, pH=4), ocetne kisline (pH=3) in žveplove kisline (pH=1).
Zanimalo nas je še, ali na sestavo vpliva tudi, če raztopina topila in brinovih jagod stoji dlje časa, zato smo raztopino žveplove kisline s pH=1 in klorovodikove kisline s pH=1 pustile stati 44 ur na zdrobljenih brinovih jagodah. To pa je negativno vplivalo na kvaliteto brinovega eteričnega olja, ker je veliko pomembnih sestavin izhlapelo.
Destilacijo smo izvedle na aparaturi za destilacijo eteričnih olj in iz 80 g brina dobile 2 mL destilata, ki smo ga brez vode prenesle v viale z injekcijskimi iglami. Destilat smo do določitve sestave na plinskem kromatografu hranile v hladilniku. Pokazal se je učinek pH vrednosti na kvaliteto brinovega eteričnega olja in najboljši rezultat smo dosegle pri destilaciji z 0,5 M HCl, kjer so bile spojine v pravilnih razmerjih. Sklepamo lahko, da je takšno brinovo eterično olje za trg primerno, saj človeku ne škoduje.

The Influence of pH on the Structure of Juniper Oil

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Our special interest lies in the area of etherical oils, while gathering ideas for our project our mantor teacher suggested that we focus on the juniper etherical oil, which is used in food industry, but it contains a great deal of harmful substances.
Through the process of distillation we got a typical commercial pattern, containing too much of sabinen and α-pinen, which have a harmful effect on human health. We found some information from the literature that the amounts of certain compounds in the juniper etherical oil are changing together with the changing of the pH value of the dissolvent.
First, we calculated the necessary volumes of the concentrated acids to prepare the solution of the HCl acid (HCl: pH= 0.5, pH=1, pH=2, pH=3, pH=4), the acetic acid (pH=3) and the sulphuric acid (pH=1).
We performed the distillation using a special device and from the 80gr of juniper berry we got 2 ml of the destillate,which we then injected without water into the small bottles. The distillate was kept safe until we determined the structure on the mass spectrometer in the refrigirator. Af effect of pH value on the quality of the juniper etherical oil appeared.
The best result was achieved at the distillation with 0.5M HCl, where the compounds were in the right ratio. We can deduce that such juniper etherical oil is suitable to use in the food industry and has no harmful effects on people.

Ali si je smiselno umivati zobe?

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V naši raziskovalni nalogi smo preizkušali učinkovitost različnih načinov čiščenja zob in ugotavljali, ali je umivanje zob sploh smiselno. S pomočjo 13 poskusnih oseb in Snyderjevega testa smo primerjali spiranje zob s pitno vodo, žvečenje žvečilnega gumija brez sladkorja, zaužitje jabolka, čiščenje zob z zobno krtačko ter čiščenje zob z zobno krtačko in zobno pasto. Pri osnovnem poskusu razlik v učinkovitosti zaradi številnih pomanjkljivosti Snyderjevega testa nismo dokazali. Na osnovi spremenjenega Snyderjevega testa in dodatnih poskusov pa smo ugotovili, da je umivanje zob vsekakor smiselno. Vse poskusne osebe, ki so si po zaužitju čokolade očistile zobe z zobno krtačko in zobno pasto, so bile namreč tako 7 minut kot 1 uro po obroku popolnoma nedovzetne za zobno gnilobo. Vpliv čiščenja je bil opazen še 3 ure po zaužitju čokolade. V primeru neočiščenih zob je bilo takoj po zaužitju čokolade 66,7% poskusnih oseb zelo dovzetnih za zobno gnilobo, v 2 urah po obroku pa so bile za zobno gnilobo zelo dovzetne vse poskusne osebe. V raziskavi smo ugotovili tudi, da je Snyderjev test v prvih 40 minutah po zaužitju hrane neuporaben za določanje razlik v učinkovitosti različnih načinov čiščenja zob. V ta namen bi bilo boljše uporabiti merjenje pH v zobnih oblogah.

Does Washing Our Teeth Make Sense?

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The aim of our study was to compare the efficiency of various types of teeth cleaning Additionally we tried to find out whether the teeth cleaning is reasonable at all. Using Snyder test in a group of 13 persons we compared flushing teeth with clean water, chewing gum without sugar, eating an apple, cleaning teeth with toothbrush and cleaning teeth with toothbrush and toothpaste. In the basic experiment we did not find any differences in efficiency of different ways of teeth cleaning due to numerous disadvantages of Snyder test. After a modification of Snyder test and some additional experiments we found out the teeth cleaning definitely has sense. All test persons who had cleaned their teeth with toothbrush and toothpaste after eating a chocolate were absolutely unsusceptible to dental caries 7 minutes and also 1 hour after the meal. 3 hours after eating a chocolate the impact of cleaning was still noticeable. In the case of uncleaned teeth there was 66,7% of test persons who were very susceptible to caries immediately after consuming the chocolate. Two hours after the chocolate-meal all test persons were very susceptible to caries. According to the result of our study we also realized that Snyder test is not useful for determining the differences in efficiency of various types of teeth cleaning in the first 40 minutes after the food consuming. In such situation measuring the pH of the plaque would be a better choice.

Optimizacija gojišč za selekcijo naravnih izolatov bakterij rodu *Bacillus*

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Bakterije rodu *Bacillus* so v naravi zelo razširjene. So v hrani, iztrebkih, smeteh, kompostnih kupih, vodi ter sedimentih. Za rast potrebujejo organski material iz rastlinskih in živalskih virov: škrob, celulozo, pektin, beljakovine in polisaharide. Hitreje rastejo pri višjih, kot pri nižjih temperaturah. Termofilni bacili celo pri temperaturi nad 50 °C. Bakterije tega rodu so zelo raznolike. Večinoma so aerobne, redkeje anaerobne in pogosto proizvajajo antibiotike. Njihova skupna značilnost je sporulacija-oblikujejo endospore, ki so zelo odporen, mirujoči štadij živih celic, ki zato preživijo tudi neugodne življenjske razmere. Nekatere vrste rodu *Bacillus* povzročajo bolezni pri človeku, živalih in rastlinah(patogeni), kvarijo pa nam tudi hrano in *lahko* povzročajo zastrupitve (kvarljivci hrane). Bakterije rodu *Bacillus* smo izolirali iz naslednjih vzorcev: iztrebki prežvekovalcev, humus, iztrebki kuncev, prst in silaža. Izolirali smo 120 izolatov in od teh izbrali 80 za nadaljnje delo. 34 izolatov je termofilnih in optimalno uspevajo pri temperaturi 50 °C, na gojišču s sojino moko, kot virom elementov N, P, S ter nekaterih mineralov, in škrobom, kot virom ogljika. Na gojišču z barvilom,bromocrezol so se štirje izolati obarvali rumeno (proizvodnja kisline), dva od teh sta bila termofilna. Za gojenje termofilnih bakterij rodu *Bacillus*v smo sestavili ter izbrali najustreznejša industrijska gojišča.

Cultivation Media Optimization for Selection of Natural Bacterial Isolates of the Genus *Bacillus*

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The bacteria of the *Bacillus* species are widespread in the nature. We can find them in food, excrements, garbage, compost piles, earth sediments and water. As a substrate they need organic material derived from plant and animal sources: starch, cellulose, pectin, proteins and polysaccharides. They grow faster by higher than lower temperatures. Thermophiles grow at temperatures under 50 °C. Bacteria of the genus *Bacillus* are very heterogeneous. They are mostly aerobic, some species are anaerobic. They are often producing antibiotics. Their common characteristic is the endospore formation. Endospores are very resistant on the environmental stress and they survive under adverse conditions. Most of *Bacilli* are not pathogenic although certain strains or species are causing diseases with the humans, animals and plants (the pathogens), they are spoiling our food and could cause poisonings (food spoilers). Bacteria of the genus Bacillus were isolated from the excrements of the ruminants, from the compost piles, from the rabbit excrements, from soil and from the silage. We isolated 120 strains and subsequently selected 80 for further characterisation and testing. 34 strains were thermophilic and their optimal cultivation temperature was 50 °C. Most suitable media for their cultivation were defined. They contain soy flour (source of nitrogen, phosphorus, sulphur and minerals) and starch (as a carbon source). Four strains were isolated on the bromocresol purple medium (yellow colonies due to their acid production) and two of them were thermophilic. For the cultivation of the thermophilic strains we designed and defined the most suitable industrial media.

Razkužila na slovenskem trgu

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Naloga ima poudarek na teoretičnem delu, vsebuje pa tudi praktičen primer dezinfekcije hleva, pregled razkužil na slovenskem trgu in anketo.
Sanitacija, ki je izraz za čiščenje in razkuževanje, je preventivna dejavnost, s katero želimo preprečiti izbruh nevarnih bolezni, ki ogrožajo zdravje živali in ljudi. Nevarne kužne bolezni, ki ogrožajo zdravje živali, lahko posredno predstavljajo tudi nevarnost za človeka. Zato živalim z dobrim programom biološke varnosti, katerega del je tudi sanitacija, nudimo zdravo in varno okolje. Zavedati se moramo, da nam le zdrava žival omogoča dobro ekonomiko vzreje.
Izbor razkužila je odvisen od veliko različnih dejavnikov, gotovo pa je eden od najpomembnejših njegova učinkovitost. Če ne uporabimo ustreznega razkužila ali uporabimo neustrezno koncentracijo razkužila, lahko mikroorganizmi razvijejo odpornost nanj. Idealnega razkužila ni, zato moramo pri njegovi izbiri upoštevati vse kriterije, v katerih ga bomo uporabljali in tako izbrali najprimernejšega.
V nalogi smo se osredotočili na dezinfekcijska sredstva, ki se uporabljajo v hlevski živinoreji in jih lahko najdemo na slovenskem trgu. Vsa smo zbrali v tabeli in o njih zapisali naslednje podatke: ime razkužila, proizvajalca, sestavo in delovno koncentracijo.
Izvedli smo tudi anketo z vprašanji o razkužilih, ki jih uporabljajo rejci. Spraševali smo o razlogih in prednostih za njihovo uporabo ter preverili, katera razkužila se najpogosteje uporabljajo na slovenskih farmah.

Disinfectants in Slovenian Market

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The sanitation is a common expression for cleaning and disinfection. It is a activity which prevents outbreak of dangerous diseases that threatens health of animals and people. Dangerous infectious diseases which threaten the health of animals can indirectly present risk for human. Therefore we provide good and safe environment for animals with suitable biosecurity programme, one part of it is sanitation. We should be aware of the fact that only healthy animal enables profitable production.
The choice of disinfection depends on a lot of different factors but certainly one of the most important is its efficiency. If we do not use a proper disinfectant or use unsuitable concentration, microorganisms can develop resistance to it. Unfortunately, ideal disinfectant does not exist. We should choose the most appropriate considering all the criteria, especially the way of use.
In our research we geather informations about disinfectants which are used by slovenian farms. The list of them consists of following data: name and producer of disinfectant, its structure and appropriate concetration.
We have also made a survey. We questioned which disinfectants are used by slovenian farmers and reasons and advantages of their use. From this data we have found the most common used disinfectants.

Seznam avtorjev / Author Index

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