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SWISS PHARMA SCIENCE DAY 2010

Swiss Pharma Science Day reaching its post-neonatal state at its third anniversary

Prof. Dr. Rudolf Brenneisen, University of Berne, President Scientific Advisory Board, Swiss Academy of Pharmaceutical Sciences (SAPhS)
Prof. Dr. Gerrit Borchard, University of Geneva, University of Lausanne, School of Pharmaceutical Sciences (EPGL)

What a "Beautiful Day" of pharmaceutical sciences at the Inselspital in Berne! On September 8, to the sounds of the U2 song, more than 120 scientists, most of them in their Ph.D. student stage, from all academic pharmaceutical institutions in Switzerland gathered for the Third Swiss Pharma Science Day. In addition, as the president of the hosting Swiss Society of Pharmaceutical Sciences, Prof. Hans Leuenberger was ready to point out in his welcoming speech, also scientists from Spain and Latin America, representing the Iberoamerican-Swiss Centre for Development of Dosage Forms (CISDEM), had joined. Prof. Leuenberger also thanked the University of Berne for their readiness to host the Science Day already for the third time.
As the host, Prof. Hugues Abriel, Director of the Dept. of Clinical Research pointed out the importance to train smart young scientists to be able to develop smart drugs for the future, and acknowledged the opportunity for the next generation of pharmaceutical scientists to talk to their peers and senior scientists during the Swiss Pharma Science Day. He promised to present results from the National Centre of Competence in Research (NCCR) TransCure initiative on membrane transporters as new targets at the next Swiss Pharma Science Day in 2011.

Member of the organizing committee, ready to start (Dorothea Lesche, Jelena Hasanova, Prof. Rudolf Brenneisen, Dr. Christian Lanz).

Dr. Felix Wüst, SWISS PHARMA and Philippe Tschopp, secretary of SSPhS and organizing committee.

Audience hall slowly filling up.

Addresses of welcome

Prof. Dr. Hans Leuenberger, President SSPhS
Prof. Dr. Hugues Abriel, University of Berne, Director Dept. of Clinical Research

Prof. Hans Leuenberger, President Swiss Society of Pharmaceutical Sciences (SSPhS).

Prof. Hugues Abriel, University of Berne, Director Dept. of Clinical Research.

Lecture 1: Keynote Lecture

Dr. Ulrich M. Grau, former CEO Lux Biosciences Inc., UMG Consulting, Long Island City, N.Y. USA: "Success factors in pharmaceutical R & D – Big pharma vs. biotech"

The scientific part of the day started with the keynote lecture by Dr. Ulrich Grau on vital successive factors in pharmaceutical research and development, comparing organisational structures in big pharma and biotech. His speech being based on case studies from his own career from scientist at Hoechst contributing to the development of the basal insulin Lantus®, through member of the executive board at BASF Pharma/Knoll, to his recent position as founder and CEO of a biotech start-up, Dr. Grau pointed out the weaknesses and strengths of the types of organisation he worked in. One of his conclusions was that the creation of small entrepreneurial-minded units in large organisations is beneficial for the development of novel drugs and therapeutical approaches. The full text of Dr. Grau's speech is also found in this issue of SWISS PHARMA. (Text of lecture in full length on p. 7).

Dr. Ulrich Grau, former CEO Lux Biosciences.

Lecture 2: Pharmacology

Prof. Dr. Jürg Gertsch, University of Berne, Institute of Biochemistry and Molecular Medicine: "Drug discovery in the endocannabinoid system: High hopes for new medicines"

Cannabinoids may not merely have a function as a lifestyle drug, as Prof. Jürg Gertsch of the University of Berne pointed out in the second lecture of the morning. Recent findings have shown evidence for the existence of an endocannabinoid system (ECS), whose receptors are not restricted to neurons, but are expressed in many tissues. The ECS may also be involved in immune system activation, as its receptors have also been found in macrophages. Starting from natural sources, several ligands have been synthesized and tested for therapeutic approaches, such as in pain management, inflammatory diseases in skin disorders and bone degradation. Small anecdote: the suggestion that endocannabinoids are also contained in chocolate, as one paper in Nature in 1996 claimed, was not confirmed.

Prof. Jürg Gertsch, University of Berne.

Lecture 3: Pharmaceutical Biology

Prof. Dr. Hermann Stuppner, University of Innsbruck, Institute of Pharmacy: "Integration of in silico methods to identify bio-active natural products"

The last presentation of the morning was given by Prof. Hermann Stuppner of the University of Innsbruck (Austria) on the identification of bio-active natural products. Using the Dioscorides and traditional Chinese medicine (TCM) databases, among others, it appears possible to identify pharmacologically active compounds from within the pool of natural products whose interaction with their respective receptors has already been optimized through evolution. Various (virtual) screening strategies, based on in silico tools such as pharmacophore modeling, docking experiments and parallel screening, are deemed indispensable tools for natural product drug discovery.

Prof. Hermann Stuppner, University of Innsbruck, acknowledged by Prof. Rudolf Brenneisen, moderator and organizer of SPhSD.

Poster session

During the lunch break, 52 posters were discussed and examined to the tunes of *Read your mind* by The Killers. Like the previous year, awards were given to three outstanding poster presentations, and one poster was given a special award due to its originality.

Prof. Ulrich Honegger, fellow 2009 and vice-president of SSPhS, and Prof. Kurt Hersberger, University of Basel.

Prof. Stefan Mühlebach, fellow 2009 and vice-president of SSPhS and PD Dr. Hans W. Schmid, fellow 2010 of SSPhS.

Academia of the University of Geneva: Prof. Muriel Cuendet and Prof. Gerrit Borchard, organizer of SPhSD.

Daniel Allemann, installing mobile drug testing lab.

Poster presenter Tasqiah Julianti, University of Basel.

Lecture 4: Pharmaceutical Technology

Dr. Sonoko Kanai, F. Hoffmann-La Roche Ltd., Basel: "High concentration protein formulations"

In openening the afternoon session, Dr. Sonoko Kanai, formulation scientist at F. Hoffmann-La Roche Ltd. (Basel), gave an inside view of her various activities at what she referred to as the "Roche University", which is "not a bad place after all". Mostly focusing on the formulation development of therapeutic antibodies, her first experiences in a "real" industrial position (after a post-doc at Genentech) also included dealings with administrative and regulatory side of drug development. Especially the PhD students and post-docs in the audience about to join the pharmaceutical and biotech industry appreciated the lively presentation of Dr. Kanai very much.

Dr. Sonoko Kanai, F. Hoffmann-La Roche Basel.

Lecture 5: Clinical Pharmacy

Prof. Dr. Ursula von Mandach, University Hospital Zurich, Dept. of Obstetrics: „Possibilities and limits in the clinical research for pharmacists at the university"

Following up, Prof. Ursula von Mandach, clinical pharmacist and head of the perinatal pharmacology group at the University Hospital Zurich, introduced her view of pharmaceutical sciences as a tree of life, which needs to be climbed with fascination, patience and, last not least, a concept. The latter refers to what one plans on doing with the versatile education and training received by pharmacy students. In the case of Prof. von Mandach, it lead to her interest in acting in a clinical setting as a pharmacist, to find solutions and offer advice in perinatal pharmacology. This service is supported and supplemented by her active research in placental drug transport and permeability of drugs, and the influence of nutrition in pre-term neonates. As a prerequisite for interdisciplinary cooperation in individualized medicine, however, clinical pharmacists must experience sufficient empowerment, which is still often lacking.

Prof. Ursula von Mandach, University Hospital Zurich.

Lecture 6: Analytics

Dr. Norbert Baume, CHUV / Swiss Laboratory for Doping Analyses, Lausanne: „The lab behind doping tests: Current challenges and perspectives"

Dr. Norbert Baume of the Swiss Laboratory for Doping Analyses (LAD, Lausanne), rounded up the day by presenting on the regulatory and analytical challenges in doping testing today. As sport may be defined as a physical activity following rules and fairplay, doping, that is the enhancement of the physical abilities of the athlete by medical means, is contradicting this the very spirit of sports. To address advances in the development of doping agents, which oftentimes are identical to endogenous substances, new analytical techniques are being developed, and their sensitivity of detection enhanced. As a service to the sports community, the World Anti-Doping Agency (WADA), with their European headquarters located in Lausanne, is releasing every year a list of forbidden substances, and substances which are not allowed in certain sports.

Dr. Norbert Baume, Swiss Lab for Doping Analysis Lausanne.

Recognitions and Awards

Prof. Dr. Gerrit Borchard and PD Dr. Hans W. Schmid New Fellows of the Swiss Society of Pharmaceutical Sciences (SSPhS) and new Members of the Swiss Academy of Pharmaceutical Sciences (SAPhS)

After a short coffee break it was time for the recognitions and awards. Two outstanding scientists, Prof. Gerrit Borchard and PD Dr. Hans W. Schmid were honored with the Fellowship of the Swiss Society of Pharmaceutical Sciences, and appointed members of the Swiss Academy of Pharmaceutical Sciences.

Prof. Dr. Gerrit Borchard, pharmacist, was nominated as Fellow by the Swiss Society of Pharmaceutical Sciences (SSPhS) and Member of the Swiss Academy of Pharmaceutical Sciences (SAPhS) for his substantial scientific contributions to Biopharmaceutics in academia and industry, as well as his outstanding contribution allowing to realize and consolidate the Swiss Pharma Science Day (SPhSD).
PD Dr. Hans W. Schmid, pharmacist, Private Docent at ETH Zurich and formerly with Cilag AG, has been elected as Fellow of SSPhS and as Member of the SAPhS for promoting entrepreneurship and industry-university cooperations. Dr. Schmid has also been the General Secretary of SSPhS during the early years of the society.
The Swiss Society of Pharmaceutical Sciences, and its Scientific Council, i.e. the Swiss Academy of Pharmaceutical Sciences, are very proud to count Prof. Borchard and PD Dr. Hans W. Schmid among their ranks.

Prof. Gerrit Borchard, fellow 2010 of SSPhS.

PD Dr. Hans W. Schmid, fellow 2010 of SSPhS.

Poster Awards

1st prize
Stefanie Zimmermann
University of Basel, Dept. of Pharmaceutical Sciences, Institute of Pharmaceutical Biology
Poster P-3

Stefanie Zimmermann, University of Basel, awarded 1st SSPhS prize by Prof. Hans Leuenberger.

2nd prize
Kathrin Fuhrmann
ETH Zurich, Institute of Pharmaceutical Sciences
Poster P-25

Gregor Fuhrmann (award winner Kathrin Fuhrmann absent), ETH Zurich, receiving 2nd prize by Vroni Jakob, PharmGZ.

3rd prize
Philipp Walter
University of Basel, Dept. of Pharmaceutical Sciences, Pharmaceutical Care Research Group
Poster P-22

Philipp Walter, University of Basel, awarded by Dr. Stefan Fritz, president AKB Foundation.

Special prize
Daniel Allemann
Pharmaceutical Control Laboratory, Office of the Cantonal Pharmacist Berne
Poster P-46

Dr. Hans-Jörg Helmlin, Pharm. Control lab Berne, receiving special prize from Prof. Rudolf Brenneisen (award winner Daniel Allemann absent).

The poster awards, being sponsored by the Swiss Society of Pharmaceutical Sciences (1st prize and special poster award), the Pharmaceutical Society of Zurich (2nd prize), and the Foundation of Bernese Pharmacists ("AKB-Stiftung", 3rd prize), were selected based on their scientific quality and clarity of presentation.
The jury voted unanimously to award the poster contribution of Stefanie Zimmermann the first prize, a cooperative project of the Institute of Pharmaceutical Biology at the University of Basel and the Swiss Tropical Institut, titled "Cynaropicrin is active against African sleeping sickness in mice". Kathrin Fuhrmann, of the Institute of Pharmaceutical Sciences at ETH Zurich was awarded the second prize for her presentation titled "Cross-linkable polymers for nanocrystal stabilization". The third prize was awarded to Philipp Walter (Pharmaceutical Care Research Group, University of Basel), who presented on "Combination of pharmacological biomarkers and compliance monitoring to detect contributing factors to drug resistance – a study design". The project was done in collaboration with The Compliers Group of DSM TCG in Urmond (The Netherlands).
This year, the jury decided again to award a special poster prize for a fourth outstanding contribution. Daniel Allemann's (Pharmaceutical Control Laboratory Berne) presentation of "Party drugs testing on the dance floor: equipment, methods and results" was chosen for this award. His poster was accompanied by an exhibition of the actual mobile lab equipment used to derive the data presented.

Thanking … and Invitation to the 4th SWISS PHARMA SCIENCE DAY on Wednesday August 31, 2011

The third Swiss Pharma Science Day ended with drinks and snacks at the beautiful setting of the House of the University. The organizers would like to thank all speakers for their excellent presentations, and the Medical Faculty of the University of Berne as the host of this event. The Verlag Dr. Felix Wüst AG Küsnacht ZH, AKB-Stiftung, the Pharmaceutical Society of Zurich (PharmGZ), pharmaSuisse, Vifor, and Galexis are recognized for their continued financial support. The organizers are looking forward to welcome young pharmaceutical scientists to the fourth edition of the 4th Swiss Pharma Science Day in 2011, again in Berne.

Relaxing after SPhSD 2010 (Proff. Gerrit Borchard, Hans Leuenberger, Matthias Hamburger, Ulrich Honegger).

Organizers of the SPhSD together with their spouses Christiane Borchard and Francesca Vollenweider (photographer).

Small talks between Proff. Stefan Mühlebach, Ursula von Mandach, Kurt Hersberger and Vroni Jakob.

Prof. Gerrit Borchard, Geneva
Prof. Rudolf Brenneisen, Berne

◆

3rd SWISS PHARMA SCIENCE DAY 2010 (www.sgphw.ch), Berne, 3rd September 2010 (Keynote Lecture)

Success factors in pharmaceutical R&D – Big pharma vs. biotech

Dr. Ulrich M. Grau, former CEO Lux Biosciences, Inc., UMG Consulting, Long Island City, N.Y., USA

Remember the Club of Rome? Almost 40 years ago the book "Limits to Growth" was published. Systems dynamics was utilized to model the impact of continued growth of the world population and of the consumption patterns of natural resources. The study predicted that, if the then recognized patterns of resource utilization and growth would continue unchanged, the earth would strike its limits within a century [1]. At the time the view was quite unwelcome and controversial and the most prominent counterargument was that the markets and yet unknown technologies would provide solutions for both the effects on resources and on the environment. However, the study suggested not only relying on technology, but changing behavior towards what is now called "sustainable development". Thus, the study's deeper message wasn't doomsday; the warning was followed by the optimistic belief that it was possible to change course and that mankind can be mobilized and motivated for that task.

What does the Club of Rome study have in common with pharmaceutical R&D?

Picture the consumption of food and industrial goods as the R&D dollars, and picture the natural resources as the output of new products, and picture the impact on the environment as the strain in the health care systems to pay for innovation, and you have described the strategic dilemma of the R&D based pharma and biotech industries. In numbers: The US biopharmaceutical industry has spent $ 65.3B in 2009, more than double the level spent a decade earlier ($ 26B) [2]. Meanwhile, the output as measured by the US approvals of new molecular entities (NME's) and biologic products has declined, or at best leveled off: 21.7 as the most recent 3-years average, compared to 35 a decade earlier [3]. What is more, approximately half of new approvals originate from biotech companies, while only 12% of the 2009 R&D spending came from venture capital sources [4]. However, approval no longer equates market access; in most Health Care Systems "Proof of Relevance" is required for formulary inclusion and reimbursement, making it very difficult to recoup the R&D investment. What we're observing in the pharma industry is a continued trend towards consolidation, and the more recent emphasis among the big players on non-R&D strategies such as commercial growth in emerging markets and diversification into medical devices, generics or consumer products. Longer-term, however, the mandate to R&D is clear: do a lot more with a lot less, and make sure the new products add real value that is recognized, and paid for, by the health care system.

The drought in new products has many roots:

- "low hanging fruit", partially discovered by serendipity and with modest effort, have largely been harvested;
- the largely futile but enormously costly attempt to scale and "industrialize" the drug discovery process with the help of genomics and high throughput screening;
- disturbances caused by the consolidation of the industry usually accompanied by the need for cost synergies and pipeline pruning;
- the pursuit of the blockbuster model, i.e. the convergence towards a limited set of "large" diseases and the inherent redundancy in the industry as a whole, and the associated need for very large studies and high safety standards to gain approval, often resulting in poorly differentiated products;
- overconfidence in the true state of our knowledge vis a vis target identification in complex biological systems, leading to more, rather than less, attrition;
- the slow adoption of large collaborations, networked R&D and strategic outsourcing;
- the slow adoption of biologics as major source of new products;
- the slow adoption of products for orphan diseases as major source of growth;
- the slow adoption of diagnostically response guided therapies, often referred to as "personalized medicine";
- sharply elevated approval and safety standards;
- organizational complexity and cultural misfit.

The good news is that these have been recognized by many and measures are in progress to change course. The bad news is that inertia, short term pressures and short term thinking are very difficult to overcome. Still, patients are waiting for us to sort things out quickly and provide new medicines for the many untreatable or poorly treatable diseases.

Here I will highlight the organizational and cultural aspects that are among the hardest to overcome when promoting change. Rather than making this a theoretical exercise, I will try to draw lessons learned from real life stories experienced by someone who has been immersed in a very large global organization, a mid-size global organization, a public US biotech, and a private biotech startup.

1981–86: Hoechst

In the early 80's Hoechst was number one among global pharmaceutical companies. Its greatest successes were derived from strong medicinal chemistry coupled with biochemical and pharmacological screening. It had a strong research culture and its senior management rose all through the ranks of R&D.

I joined the biochemistry research center as a young scientist at a time when genetic engineering and molecular biology were emerging. Among other major pharma companies Hoechst was in a unique position with regards to biologics as one of the original licensees of the University of Toronto for insulin. I got involved in engineering improved insulins, first leading to human insulin derived from e.coli from a proinsulin-like precursor, and then leading

to Lantus®, today's best-selling long-acting, engineered basal insulin (2009 revenues $ 4.4 B).
The conditions to do research at Hoechst were principally good, and the access to expertise e.g. in a department called Applied Physics with its state of the art equipment and experts was excellent. To my amazement, these resources were hardly utilized and Central Research was later converted into a service provider. However, within pharma R&D the scientific excellence, the desire to succeed and be first, and the self-motivation and sense of being part of a great team or major collaborations left much to be desired. Each lab was equipped with two or three technical staff, who tended to adhere strictly to an 8-hour workday. Enormous amount of time was spent for lengthy meetings and presentations. Internal presentations were of widely varying quality and scientific discourse and healthy peer pressure were virtually absent. Life in R&D became routine, spanning for many an entire 30+ year career. There was no senior career path in R&D other than management. Not surprisingly, the best people left the labs after a few years, typically through promotion.
With few exceptions, preclinical Research and clinical Development were silos. Clinical Research felt that compounds lacked originality and were "thrown over the fence", and they were improperly faulted for failures. Conversely, preclinical researchers complained that all their great work was destroyed by disingenuous clinical development.
At one time a new head of research joined the company who felt there was nothing to be gained trying to tweak insulin but the time had come to search for molecules that modulate gene regulation and allow expression and release of insulin, which could be easily formatted in a high-throughput screen. Along similar lines, when we had successfully isolated the first mg insulin from coli our colleagues at Behring who isolated large proteins, such as factor IIX from blood, told us bluntly that proteins of this magnitude could never be made through fermentation, and, hence, saw no need to invest in this type of research.
Meanwhile, Hoechst invested $ 50M in Howard Goodman's lab at MGH in Boston, which was supposed to help catching up with the science – in hindsight a great move. Sadly, though, Hoechst missed to take advantage of one of the most significant discoveries coming out of the group at the time: Brian Seed wrote the patents on receptor-Fc fusion proteins, which includes what is now Enbrel®.
Within a few years, Hoechst had fallen from the leading position among global pharmaceutical companies, largely because the company was insular and unproductive in its R&D, and did not seize opportunities to grow and build a major presence in the US. Before long Hoechst was drawn into mergers from weakness and eventually Aventis, itself the product of a merger between Hoechst Marion Roussel and Rhone Poulenc Rorer, was taken over by Sanofi-Synthelabo. Behring has been sliced up into 3 pieces that are now part of other conglomerates.

The lessons learned here are:

- **Although deep in resources, Hoechst somehow could not continue a stellar track record of groundbreaking discoveries that included diuretics, antidiabetics, analgesics, peptide hormones or antiinfectives.** I believe the company was pursuing too many leads, became too complex in its processes, departments pursued their own pet projects, and the middle management failed to pull together. Upper management was proud of their past achievements and the commercial success of the present, lacked the courage to execute an acquisition opposed by local US management and failed to inject excellence, desire and momentum into the organization, and R&D became for many an 8-hour a day affair.
- **It is good to be humble, but decisive.** It appeared that the Hoechst leadership saw little need to cooperate with the outside world, did miss the emergence of the US as the leading pharma market and driver of innovation, and generally thought that their way of doing things was second to none. On the other hand, management appeared to be passive; remember: no decision is also a decision.
- **New technologies need to be adopted thoughtfully.** It is generally very difficult to reduce visionary ideas to practice, but is, likewise, unwise to dismiss their potential. The answer is a risk balanced project portfolio. Academic/biotech collaborations are a way to mitigate these risks.
- **Collaborate and focus on translational science.** With mechanisms and pathways mostly explained in naïve cartoons, the search for clinical correlates through smart translational research is critical. Research has not succeeded with a preclinical finding, but only with proof of concept in man.

1993–1999: Hoechst Marion Roussel/Aventis

I got involved in 3 mergers: in the first and second being charged with integrating the worldwide research footprint in immunology (at the time of the second merger located at 6 sites in the US, Europe and Japan) which was consolidated into two sites; in the third merger charged with integrating the worldwide R&D organizations between HMR and RPR to form Aventis. The latter M&A was a major transformation in the sense that with it the entire Hoechst Group – chemicals, fibers, polymers, technical ceramics, specialty chemicals, engineering, pharma, agro, industrial gases, etc – was dissolved, all other businesses spun out or sold, and Aventis became a pure Life Science Concern with pharma, agro, vet and diagnostics businesses.
That transformation was designed to transition from the P/E multiples of a chemical and commodity company to those of a pharma company, and as such would make Hoechst less vulnerable to takeovers. The flaw in this strategy was that the market did not look at Aventis over night as a different company. For years, its stock traded well below the peer group and eventually it became prey to Sanofi, despite its much larger size.
On the pharma side, there was the stress related to M&A, such as the uncertainty which projects would become part of the combined pipeline, which sites would be maintained and which would be closed. The loss of R&D productivity was immediately visible.
Based on the documented attrition models at the time, we modeled for HMR with an R&D budget of approximately $ 2B an organization that would produce 3 NDA's per year of average peak revenue of $ 400M. Incidentally, this led to a massive buildup of capacities in Lead Optimization (the phase from selection of a preclinical lead compound through proof of concept in man), tailored to produce some 25 IND's per year, a goal that was never achieved.
When Aventis was formed, at roughly twice the size, it was clear from the beginning that this approach would be nonsensical, as the company would have to generate some 50 IND's and 6 NDA's per year. In a stroke of genius we decided to keep the number of projects as before, but double their market potential. Hence, the R&D organization was now charged with creating 3 products of $ 800M revenue potential per year (at the time, the average proprietary pharma product produced about $ 275M per year in worldwide revenues). In addition we determined that through "breakthrough" initiatives we'd find ways to beat the reported attrition rates. It is rather evident that cranking out blockbusters on a regular basis, and at the same time beating benchmark failure rates consistently was mission impossible.

The lessons learned here are:

- **Mergers may be inevitable, but there are diseconomies of scale in R&D.** The price to be paid with mergers is loss in R&D productivity. Usually a select set of projects that are deemed of critical importance – often those close to submission or approval – are being ringfenced and the teams continue work with full

speed. However, pipeline pruning, eliminating overlaps, process re-engineering and setting up a new organization absorb a large part of the R&D organization for a considerable amount of time – typically one year or more until the changes are implemented – during which time many projects sit idle or progress very slowly.

- **Larger R&D organizations typically rely on processes and standards to manage complexities which may be toxic for creativity.** Big organizations tend to stifle creativity and build bureaucracies instead. The R&D pipeline in large pharma managed by a Portfolio Committee exceeds 100 projects, which may double with a merger, and can reach 300 projects in the largest organizations. The decision makers no longer fully understand the science or the technical or commercial risks, nor do they know all of the key scientists or developers. Processes and managerial layers become inevitable. Of the enormous depth of knowledge that is present in a large pharma organization, much is lost in unintentional communication sinkholes.
- **When R&D based, a much more nimble specialty business model ("string of pearls") may be sustainable.** As blockbusters are rare and unpredictable, ways have to be found to build businesses in therapeutic franchises with perhaps some larger as well as smaller products. The R&D enterprise has to be much more flexible to adjust to this. Orphan products can be large sellers, but in general smaller products cannot carry the huge overhead costs; biotech can be very efficient in this domain.

2000–2001: BASF Pharma

Having completed the global R&D integration plans, I left Aventis and joined BASF Pharma/ Knoll as President of R&D. The company had, among others, a state of the art biologics R&D facility in Worcester, MA, which most prominently came up with adalimumab (Humira®). That product was about to enter phase 3 clinical development. However, the former head of R&D had little influence over the R&D centers in the US, neither the clinical development which was the turf of the President US Commercial Operations, nor the Biologics Manufacturing facility, which reported into Central Research at BASF in Ludwigshafen.

The net result was that when I looked into the development plan for adalimumab, I realized that there were actually three. For example, the US clinical studies were to be conducted with weekly injections, while the European phase 3 studies were to be conducted with bi-weekly injections. No one was willing to give up their positions. Within two months I managed to globalize the R&D organization and had the team harmonize the development plan into a single global plan. In addition, I appointed a very senior and experienced person to direct the global team, reporting to a SVP Global Programs, who reported to me.

I also participated in a weeklong retreat to define the development plan into considerable detail, such that it eventually contained some 4,000 line items. In the beginning of 2000, we then determined that the target NDA submission date was March 28, 2002. Major investments, such as the expansion of our manufacturing facility for about $ 80M, were all tied to this very date. As is known Abbott acquired BASF Pharma in 2001, and they followed my recommendation to ringfence the team in New Jersey rather than "absorb" the program into Abbott Park. The Humira NDA was actually submitted on March 25, 2002 and approval was obtained in Dec. 2002. Abbott reported revenues for Humira of over $ 6B in 2009.

The lessons learned here are:

- **BASF Phama had a near-ideal size from an R&D perspective.** It had global reach with sites in the US, Europe and Japan, had resources that were major, but forced focus, and was still nimble enough to allow for flexibility. As such it fell into the league of R&D based specialty pharma or large biotech companies, such as Celgene or Actelion, a group that has done remarkably well in recent years. However, the company was sold and is now part of Abbott.
- **Organizations need to support the business purpose.** The implementation of a global R&D organization that was hungry for success was critical. People like to build empires and define themselves by the size of the empire and the span of control. One of my former bosses said: it's not important what you control; it's important what you contribute.
- **Products are the lifeblood in our industry, not processes.** Although new products are critical for the industry, few organizations allow truly empowered teams and structure the organization around programs. In addition, complex organizations lead to collective indecision and lack of momentum. Innovation and progress are achieved in small groups that are empowered.
- **Diligent and detailed planning is needed.** Especially in late stage development and approaching NDA submission, detailed planning is business-critical. If it isn't planned, it won't happen. The exercise of putting together a plan is in itself enlightening to team members, and the deliverables contain a personal commitment to the team, and create significant peer pressure if not achieved.

2006–2010: Lux Biosciences

We founded Lux in 2006 and the company was specialized on inflammatory diseases of the eye based on the medical needs, the specialty nature of the markets, the manageable risks, the founding team's background in immunology and the founding CMO's training as ophthalmologist and uveitologist. The molecules themselves were in-licensed from companies exploring the same drug in other diseases, while Lux secured exclusive rights for ophthalmic use. In doing so we focused on innovation, such as patented first-in-class products. Furthermore, we sought to build a staged portfolio allowing for several shots on goal underpinned by proprietary technologies for ocular delivery. And finally, we adopted a networked organizational model with an internal team of only 23 and a network of consultants and service providers. Only with that model could we advance from in-licensing to the clinic in as little as 9 months. Instead, we focused on managing networks efficiently, and negotiated carefully contracts with service providers.

We've raised a total of $ 99M of venture capital. Comparing the capital efficiency with what I experienced at Aventis, I can easily claim that we did late stage development at a fraction, at least 1/5th the cost compared to big pharma. (Remember: the mandate in pharma R&D is to do a lot more with a lot less.)

We conducted our clinical trials in 3 continents, and 7 countries (North America, Europe and India), and having feet on the ground in countries where the trials are conducted, as well as for interaction with the European health authorities, was the reasoning for setting up a small subsidiary in Frankfurt.

Culturally, the place could be described as high-energy, strong sense of ownership and urgency, and committed to meeting targets, data-driven, extremely focused and highly transparent. We worked essentially task-driven and solved problems. Underlying this we had a detailed project plan – along the lines described for adalimumab – and we were fully focused on flawless execution of the plan. By and large we have been able to meet our goals: within less than 4 years since establishing the company, we have moved our lead program in uveitis from inception and no data in ophthalmology through pivotal phase to the point where the NDA and MAA were submitted simultaneously in early 2010. This was a daunting task for a small team: first submission of this new molecular entity and compilation of an electronic file of some 20 GB size (roughly 1 million pages).

Now, there is a twist to what would otherwise be described as a spectacular success story: the FDA determined that one of two pivotal studies did not demonstrate adequate efficacy (partially due to design features that were without precedent), and the company is now conducting one further pivotal study. The approval process in Europe is still active and might result in a positive outcome.

The lessons learned here are:

- **Biotech is clearly the place for creativity, innovation, commitment and speed.** Everyone including the CEO was rolling up the sleeves and got their hands dirty. Very little time was wasted in nonsensical meetings or reviews. Decisions were taken in a timely manner and implemented. The work was fun, the team had a sense of achievement and team members learned and grew as they assumed increasing responsibility and took ownership and pride in their work. The ownership was also literal and allowed everyone to participate in the potential success. This more than compensated for the fact that the expectations were high, long hours were the norm and the personal risk was imminent.
- **The pressures in Biotech are high, carry personal risks and can lead to shortcuts and cause deficiencies.** The continuous struggle for funding sometimes overshadows the work. Capital efficiency is a must in Biotech. Cash from VC sources is extremely costly and even minor deviations from the "investment thesis" are painful. As these investment theses are built on a set of assumptions and projections at the beginning of a program, e.g. without knowledge of the detailed regulatory requirements, deviations are the rule, not the exception. Shortcuts to save money often become boomerangs.
- **It is important to associate with the right investors and achieve alignment.** Venture Capital firms are in the business of generating maximum return for their investors whose funds they manage. Biotech management typically aspires to creating great products and a great company. In many cases these goals lead to the same result. However, there may be situations where great programs essential for product flow are hibernated because the "value inflection point" isn't in the right time window for an exit. Likewise, external influences may surprise, such as the recent financial crisis which caused panic especially among those VC firms trying to raise new funds, suddenly unable to do so. This created enormous pressure on every private biotech company in their portfolio to sell itself. With the IPO market dried up, the result was a buyers' market as pharma companies waited and took advantage. Such issues can become the subject of discontent. So, the key lesson here is to associate with the right investors and Board members, be clear about the true intentions and the best means to reach consensus at the outset, and communicate very well.

In conclusion, over the course of my career I have been involved in large global pharma, mid-size global pharma, public biotech and private biotech. No single element is suitable to solve all the challenges pharma R&D poses. In the end, success is determined by the organizational setup, the leadership, the product portfolio, by the people involved and by adequate resources.

It has become common wisdom that research is a small-group process, relies on frequent informal interactions and therefore it is an advantage if research teams are co-located; individual effort often produces the sparking idea and incentives such as company stock with major upside are clearly motivating. Furthermore, as new data are generated or published constantly these individuals need great flexibility. Bureaucracies or heavy-handed management are frustrating and toxic.

However, I see the pendulum swing back from biotech as the major source of research innovation because of two trends: First, large pharma companies have amassed technologies and provide sophisticated tools, and in realizing the challenges have taken measures to create biotech-like structures in order to foster creativity. Second, the venture funding climate has changed. Most funds prefer clinical stage projects. The path from the lab to proof of concept in man is simply too long as deals prior to proof of concept are more and more difficult. There are notable exceptions from that rule, primarily enabling technology platforms.

Which brings me to the paradox in today's Biotech: although big pharma would passionately argue that late stage development, regulatory approvals, supply chain management and transitioning of products into the marketplace are their core strengths, require muscle and usually are less dependent on small group creativity but benefit from strong processes and project management, that is exactly the domain into which Biotech has been moving. Indeed, in my view Biotech can be far more efficient and effective so long as the indication is specialized and trials of not more than perhaps 1000 patients are needed. That applies to the majority of development candidates and addresses at least one critical challenge of today's pharma R&D, namely its extraordinary cost and poor productivity.

One critical fact remains: the emergence of the biotech industry and its successes has led to a new pharma R&D landscape, in which collaborations and interdependencies are the norm. This necessitates a narrowing of the cultural gaps and a meeting of the minds. Like the Club of Rome, in pointing out the challenges and high risks associated with pharma R&D, I'd like to close with an optimistic note. The medical need in many indications is still high, the promise of new medicines is highly appealing, the R&D enterprise in large and small organizations is adjusting and bright people are still being attracted. However, in a Darwinian environment, we will continue to see stars rising, and others disappear and past success is not a guarantee for future success. It never has.

About the Author

Ulrich M. Grau, Ph.D. has nearly 30 years of experience in the biotechnology and pharmaceutical industry. His expertise in the healthcare field includes general and R&D management, business development, corporate strategy, merger integration and alliances, and the development of new products and technologies for a variety of diseases.

Ulrich served in a variety of roles in a very large pharma company (at Hoechst Marion Roussel and Aventis as research scientist, head of a research unit, head of a Therapeutic Domain, General Manager of a country affiliate, and Senior VP Global Late Stage Development), a mid-size pharma company (at BASF Pharma/Knoll as President Global R&D), a public US Biotech company (at Enzon Pharmaceuticals as Chief Scientific Officer), and in a private US Biotech company (at Lux Biosciences as founder, President and CEO). This unique set of experiences allows him to extract authentic conclusions about success factors and cultural aspects in pharma and biotech R&D.

References:
[1] Donella H. Meadows, Dennis L. Meadows, Jørgen Randers, and William W. Behrens III, The Limits of Growth, 1972
[2] Burrill & Co, PhRMa member survey; PhRMa website: http://www.phrma.org/key_industry_facts_about_phrma
[3] FDA Division of Business Analysis and Reporting, Office of Business Process Support http://www.fda.gov/downloads/Drugs/DevelopmentApprovalProcess
[4] Battelle Study prepared for BIO, May 2010

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SWISS PHARMA SCIENCE DAY 2010

Poster Session – Abstracts

P-1

Detection and Isolation by MS-Coupled Preparative HPLC of Lysophosphatidylcholine Derivatives from Goji Berries *(Lycium Barbarum)*

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Introduction: Goji berries (wolfberries) *(Lycium barbarum* L., Solanaceae) are a traditional food and medicine in East Asia which have become increasingly popular in Europe and North America over the last years. Goji berries and juice are being sold as health food products and praised in advertisements and in the media for well-being and as an anti-aging remedy. There have been increasing concerns about the quality of goji products with respect to pesticide contamination or possible adulteration, so that reliable quality control procedures are, therefore, urgently needed.
Aims: Identification of a group of late-eluting compounds devoid of UV absorption, which could not be assigned to any known constituents of goji berries.
Methods: An approach based on MS-coupled preparative HPLC was used for the isolation of the UV-inactive compounds. The experimental setup consisted of a HPLC-MS instrument equipped with an adjustable flow splitter and an additional pump delivering a make-up flow. Separations were performed on a semi-preparative RP-18 HPLC column (10 x 150 mm, i.d.). The compounds were identified by a combination of spectroscopic and chemical methods including ESI-MS, 1D- and 2D-NMR, and GC analysis of the acyl residue after methanolysis.
Results: Five main compounds were isolated in sub-milligram to milligram amounts from the CH_2Cl_2/MeOH 1:1 extract of the berries. They were identified as lysophosphatidyl-choline derivatives with fatty acid residues of variable length and degree of unsaturation.
Conclusions: Such metabolites have not been reported hitherto in goji berries, but a mixture of phosphatidylcholine and lysophosphatidylcholine derivatives with analogue fatty acid composition has been detected in jojoba seed meal *(Sim mondsia chinensis).* These compounds may be useful as chromatographic markers for the analysis of goji products. At the same time they may give new hints with respect to the biological properties of goji berries and products derived thereof.

Keywords: Mass-coupled preparative HPLC, goji, *Lycium barbarum,* lysophosphatidyl-choline derivatives.

P-2

Flash Chromatography on Cartridges for the Separation of Plant Extracts

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Introduction: Flash chromatography on cartridges has become increasingly popular for the rapid purification of compounds, mainly of synthetic origin. In contrast, its application for natural product isolation is poorly documented, and easy-to-use procedures for optimization of the separation conditions are lacking.
Aims: Development of empirical guidelines for the use of Sepacore® cartridges and their application to the separation of plant extracts.
Methods: Separations were performed on a Sepacore® chromatography system equipped with prepacked cartridges (Büchi Labortechnik AG). The performance of the cartridges was compared to that of classical MPLC (medium pressure liquid chromatography) glass columns.
Results: Reversed phase HPLC separations can be transposed by increasing the gradient time by a factor of 2–4. For normal phase separations, solvent compositions resulting in R_f values of 0.15–0.2 on TLC for the most lipophilic and hydrophilic constituents, respectively, should be selected as gradient endpoints. These rules were successively applied to the separation of complex plant extracts, with *Curcuma xanthorrhiza, Piper nigrum* and *Salvia milthiorrhiza* as examples of medicinal and commercial importance.
Conclusions: Sepacore® cartridges enabled a good separation of compounds with a broad range of polarity, as typically found in plant extracts. The chromatographic resolution remained, however, lower than that achieved by MPLC on columns packed with material of smaller particle size. For poorly soluble extracts, solid introduction gave better results than liquid injection. Despite lower resolution as compared to MPLC, pre-packed cartridges are an attractive alternative for the purification of extracts and crude fractions due to their ease of use and speed of separation.

Keywords: MPLC, flash chromatography, Sepacore.

P-3

Cynaropicrin is Active Against African Sleeping Sickness in Mice

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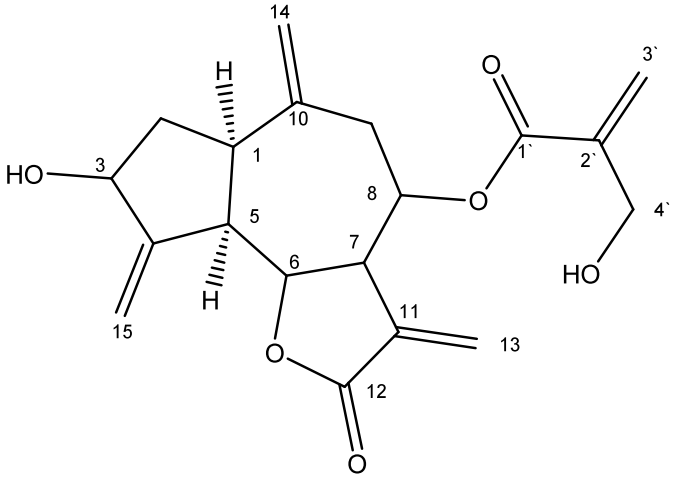
Introduction: In a medium throughput screen of 880 plant and fungal extracts for antiplasmodial, antitrypanosomal and leishmanicidal activity, the dichloromethane extract of *Centaurea salmantica*

(Asteraceae) showed strong inhibition against *Trypanosoma brucei rhodesiense,* the parasite, which causes African sleeping sickness.
Aim: To identify the substance responsible for this activity, and to test it *in vivo.*
Methods: HPLC-based activity profiling led to the characterisation of cynaropicrin, a guajanolide sesquiterpene lactone, as the active principle.
Results: Against *T. brucei rhodesiense* cynaropicrin had an IC_{50} of 0.3 µM. It was ten and fifteen times less active against *P. falciparum* (IC_{50}: 2.99) and against *T. cruzi* (IC_{50}: 4.43), respectively. A series of similar natural and semi-synthetic guaianolides were tested for preliminary structure activity studies.



Mice infected with the bloodstream form of *T. brucei rhodesiense* were treated daily with 10 mg/kg cynaropicrin i.p. for 4 consecutive days. Cynaropicrin decreased the parasitaemia by 98% compared to the untreated controls. The test animals showed a 100% survival until day 14 after infection whereas the control animals died within 12 days.
Conclusions: This is the first study of a natural product showing *in vivo* activity against *T. brucei rhodesiense.*

Keywords: HPLC-based activity profiling, guajanolide sesquiterpene lactone, *in vitro* and *in vivo* activity, *T. brucei rhodesiense.*

P-4

Characterization of Ketamine N-Demethylation by CYP3A4 and CYP2B6 Using Enantioselective Capillary Electrophoresis

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Introduction: Ketamine, a phencyclidine derivative, is used for induction of anesthesia, as an anesthetic drug for short term surgical interventions and, in subanesthetic doses, for postoperative pain relief. Ketamine is also a drug of abuse. It consists of a racemic mixture of two enantiomers, *S*-ketamine and *R*-ketamine. *S*-Ketamine has a higher affinity for the NMDA-receptor, the mu and kappa opioid receptors as well as a higher anesthetic potency compared to *R*-ketamine. Based on previous work in which the hepatic cytochrome P_{450} (CYP) metabolism of ketamine was studied qualitatively, the metabolism is suspected to be stereoselective.
Aims: The goal of this work was to study and analyze the stereoselectivity of the N-demethylation of ketamine to norketamine catalyzed by two enzymes, CYP3A4 and CYP2B6, *in vitro.* The determination of the kinetics for incubation with racemic ketamine, *S*-ketamine and *R*-ketamine was anticipated.
Methods: Kinetic studies were performed with 10 substrate concentrations of racemic ketamine (5 to 1000 µM) and single enantiomers (2.5 to 500 µM). The incubation time was 8 min. After incubation, samples were extracted at alkaline pH and redissolved in 50 µl of 5 mM tris-phosphate buffer (pH 2.5). Analysis was performed by enantioselective capillary electrophoresis using multiple isomer sulfated β-cyclodextrin as chiral selector. The obtained data were analyzed by two kinetic models (Michaelis-Menten and Hill equation) using nonlinear least square regression analysis. A paired t-test was used to determine whether a stereoselective metabolism is present at a significance level of $p < 0.05$.
Results: Incubation of racemic ketamine with CYP3A4 revealed data which, for both enantiomers, fitted better to the Michaelis-Menten model. K_m and V_{max} for *S*-norketamine formation were higher compared to those determined for *R*-norketamine. The Hill equation provided a better fit to the data obtained with CYP2B6. For both cases, statistical differences were noted. Incubation of single enantiomers resulted in higher K_m and V_{max} values.
Conclusions: Using enantioselective capillary electrophoresis and data analysis, the stereoselective kinetics of ketamine N-demethylation by CYP3A4 and CYP2B6 could be determined. Incubation of racemic ketamine and single enantiomers resulted in higher values for the kinetic parameters. This is plausible as both enantiomers with different affinity to the enzyme might interfere in the other's metabolism. Although an *in vitro* experiment is not completely comparable with an *in vivo* situation, this aspect of interaction should still be considered when chiral drugs are given.

Keywords: Capillary electrophoresis, ketamine, demethylation kinetics, CYP3A4, CYP2B6.

P-5

Identification of Individual Cytochrome P450 Enzymes Involved in the Metabolism of Ketamine and Norketamine in Man

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Introduction: Ketamine, an injectable anesthetic and analgesic consisting of a racemic mixture of *S*-and *R*-ketamine, is routinely used in veterinary and human medicine. Metabolism and pharmacokinetics of ketamine in man have not been characterized sufficiently.
Aims: An enantioselective capillary electrophoresis (CE) assay for ketamine and its metabolites in microsomal preparations has been developed and applied to the identification of individual human CYP_{450} enzymes catalyzing ketamine to norketamine and norkatemine to further metabolites *in vitro.*
Methods: The CE assay is based upon analyte liquid/liquid extraction at alkaline pH and the use of a CE buffer composed of tris/phosphate at pH 2.5 containing 10 mg/mL of multiple isomer sulfated beta-cyclodextrin as chiral selector. This approach permits the simultaneous anionic analysis of the stereoisomers of ketamine, norketamine, dehydronorketamine and hydroxylated norketamine metabolites with hydroxylation at the cyclohexanone ring. Commercially available recombinant human CYP_{450} enzymes (SUPERSOMES) were incubated with racemic ketamine or racemic norketamine and samples were analyzed after incubation time intervals of 0, 60 and 120 min.
Results: Six CYP_{450} enzymes (3A4, 2C19, 2B6, 2A6, 2D6 and 2C9) were identified to catalyze ketamine N-demethylation among which three enzymes (3A4, 2B6 and 2A6) generated norketamine stere-

oselectively. Two enzymes (2B6 and 2A6) were found to be associated with the formation of norketamine metabolites (hydroxylated norketamine metabolites and dehydronorketamine). With three enzymes (2E1, 1A1 and 1A2), no ketamine metabolites were detected.
Conclusions: The results suggest that the metabolism of ketamine in man is mainly catalyzed by CYP3A4, CYP2B6, CYP2C9 and CYP2C19. The work highlights the value of enantioselective CE in drug metabolism studies.

Keywords: Capillary electrophoresis, ketamine, norketamine, CYP, stereoselective metabolism.

P-6

Transferrin Immunoextraction for Determination of CDT in Human Serum by Capillary Zone Electrophoresis

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Introduction: Carbohydrate-deficient transferrin (CDT) is a marker of excessive chronic alcohol consumption. The high-resolution capillary zone electrophoresis (CZE) assay for CDT in which serum is mixed with an iron-containing solution prior to analysis, is an effective approach for the determination of CDT in human serum. Sera of patients with progressed liver cirrhosis, however, can have a low transferrin level and/or interferences in the β-region due to elevated immunoglobulins which prevent the proper determination of CDT by CZE. Previous work revealed that immunopurification of transferrin is the best approach to pretreat such samples.
Aims: The need of a simple and economic approach to immunoextract transferrin from human serum prompted us to investigate the manufacturing and use of an anti-transferrin spin column containing polyclonal anti human transferrin antibodies linked to Sepharose 4 Fast Flow beads.
Methods: Polyclonal anti-human transferrin antibodies were linked to CNBr-activated Sepharose 4 Fast Flow beads according to the instructions of the bead manufacturer. A spin chromatography column served as container to hold the swollen beads with the attached antibodies. Five-fold diluted serum (400 µL diluted to 2000 µL) was applied in four 500 µL aliquots with 15 min incubation of each aliquot or as batch with a 1 hour incubation at room temperature. After immunocapture, the column was rinsed with 10 mM pH 7.4 Tris/HCl buffer containing 150 mM NaCl to remove non-specifically bound proteins followed by stripping transferrin from the beads using an acidic glycine buffer. The buffer is replaced with 50 mM pH 8.4 Tris/HCl buffer containing 150 mM NaCl and the sample was concentrated on an ultrafiltration spin column prior to analysis of the extract by CZE. Lipemic sera were delipidated using a mixture of di-isopropyl ether and butanol prior to immunoextraction.
Results: The developed procedure was applied to a number of relevant patient samples and could thereby be shown to represent an effective method for transferrin extraction and concentration. CDT could unambiguously be determined in all pretreated samples. The laboratory made extraction columns can be reused many times provided that the columns are properly maintained and stored.
Conclusions: The developed extraction procedure permits quantitation of all transferrin isoforms in sera with high immunoglubulins, such as those of patients with progressed liver cirrhosis, and facilitates the determination of CDT in sera with a low transferrin content. Analysis of the flow through fraction of the sample, which is devoid of transferrin, provides information about the removed interferences and their appearance in the electropherograms.

Keywords: CDT, immunoglobulin, capillary electrophoresis, liver cirrhosis, immunoextraction.

P-7

Novel Process Analytical Concepts in Pharmaceutical Dry Milling

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Introduction: Pharmaceutical dry milling is an important unit operation for solid dosage form manufacturing. The process variables and particle characteristics should be closely monitored in order to build quality into the final dosage form. The concept of process analytical technology (PAT), since its introduction by the FDA, has been encouraging manufacturers to experiment with new approaches and technologies to better comprehend and control their processes.
Aims: (a) The objectives of this work were to implement *on-line* dynamic image analysis (DIA) and to introduce a novel *at-line* flowability analyzer in pharmaceutical dry milling ; (b) as a special aim these novel process analyzers were employed in *robustness testing* using a Taguchi design.
Methods: A conical mill ConiWitt-150™ was equipped with a DIA system XPT®-CV which consisted of a xenon flash light and charge-coupled device (CCD) camera. Powder avalanching analyzer (Revolution®, Mercury Scientific Inc., USA) was used for characterizing avalanching behavior. A 3^3 factorial design with feeder speed, impeller speed and screen size as process parameters was chosen. The response surface was calculated for different particle size measures and flow parameters using a pharmaceutical placebo granulate. Two control factors and two noise factors were considered for robustness testing (Taguchi design).
Results: (a) Impeller speed (p=0.0034) and screen size (p=0.0001) significantly affected the mean particle size at the 95% level (Fig. 1). The feeder speed, interestingly, did not exhibit a notable effect. Avalanche angle (Fig. 2) obtained from powder avalanching analysis resulted in a model goodness of fit of R^2=0.88. This flowability parameter revealed a significant interaction between feeder speed and screen size. Moreover, the avalanching values were in good agreement with flow rate through an orifice. (b) In the robustness testing design, the control factors impeller speed and screen size showed a statistically significant effect on d_{50} and d_{95}. The two noise factors, namely different granulate lots and temperature conditions, strongly affected the avalanching parameters and flow rate. Avalanche angle (p<0.0001) and flow rate (p=0.0002) were significantly influenced by the factor of granulate lots. Temperature also influenced avalanche angle (p<0.0001) and flow rate (p=0.0004).

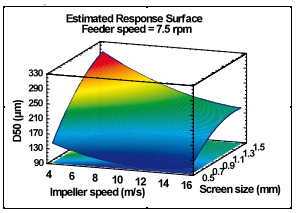

Fig 1: Estimated response surface for d_{50}

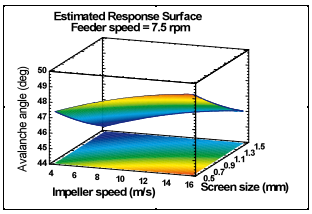

Fig 2: Estimated response surface for avalanche angle

Conclusions: The presented PAT tools in this study provided complementary information regarding the particle size and flow rate. Robustness testing results emphasized the importance to balance process factors with potential noise factors. Thus, embedding process analyzers into the design and implementing robustness testing can aid in building quality by design into the final product.

Keywords: Process analytical technology (PAT), powder avalanching, dynamic image analysis, particle size, flowability.

P-8

Functional Polymers for Arginine-Specific Protein Modification

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Introduction: Conjugation of a synthetic polymer to a protein is an elegant solution to current challenges in nanotechnology, biotechnology, and materials science. However, preparing such hybrid systems while maintaining therapeutic/catalytic activity, specific (bio) recognition, etc. is a complex undertaking. One attractive approach is to employ residue-specific reactions, which permit the introduction of polymer chains onto solvent exposed amino acid residues of a given sort. The attractiveness and popularity of this approach lies in its simplicity as it makes use of the protein's intrinsic reactivity and does not require post-modification and/or the introduction of non-natural amino acid residues. There currently exist residue-specific reactions for 8 out of the 20 canonical amino acids found in proteins. New applications demand that this toolbox be expanded so as to allow versatility in conjugate design.
Aim: The present study shows how to expand this toolbox to arginine residues. Arginine is a very interesting target amino acid, which is as abundant as lysine in nature (5.44 and 5.91% for arginine and lysine, respectively) but is less reactive, and has a lower tendency to be located at the surface of the protein. Therefore, more selective protein modification may be achieved by targeting this residue in comparison to lysine.
Methods and Results: A biologically-inspired strategy for the residue-specific modification of arginine residues with synthetic polymers will be presented. The conjugation strategy is derived from the non-enzymatic glycation of proteins *in vivo* (Maillard reaction). This reaction involves the reaction between amino groups of a protein and reducing sugars or other endogenous aldehydes or ketones. Despite the greater reactivity of lysine residues towards ketones/aldehydes, di-carbonyl reagents participating in this reaction preferentially modify arginine residues (irreversible) in favor or lysine or cysteine (reversible).
We present the thermodynamic selectivity of this reaction towards arginine and its potential for use as a conjugation reaction. Reaction conditions for achieving selective modification of arginine with a di-carbonyl compound are detailed and the stability of the formed adducts towards acidification/ basification, nucleophilic displacement, and dilution is evaluated. We then describe the synthesis of semi-telechelic polymers bearing bio-mimetic α-oxo-aldehyde groups (di-carbonyl) at their terminus. The reaction of the latter with a model protein is characterized in detail and the stability of the conjugates is examined.
Conclusions: Overall, the experiments revealed that α-oxo-aldehyde end-groups can be successfully used for the permanent and selective conjugation of polymers with arginine residues on proteins in the presence of all other amino acid residues. This reaction is highly advantageous as it can be conducted under mild conditions in the absence of catalyst and constitutes a worthy addition to the toolbox of residue-specific reactions available for preparing protein-polymer conjugates.

Keywords: Protein, polymer, PEGylation, arginine, methylglyoxal.

P-9

Accelerated Formulation Optimization for Nanomilled APIs

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Introduction: Nanomilling refers to particle size reduction of the active pharmaceutical ingredients (APIs) down to the sub-micron range. Recent advances in stirred media milling have enabled the production of API nanosuspensions with API particle sizes of 100–300 nm. Such nanosuspensions can be further processed into solid oral dosage forms by spray-drying/-granulation and tabletting or into oral suspensions. Regardless of the final dosage form, the behavior of API nanosuspensions during production, storage and further processing requires detailed investigations and optimizations.
Aims: The present work provides two case studies for the comprehensive formulation characterization of API nanosuspensions, which drive the optimization of production, storage and downstream processing attributes within the early stage of pharmaceutical development.
Methods: Compound A and B were processed into API nanosuspensions by stirred media milling at constant specific energy input and using 10% (w/w) API along with varying stabilizers in aqueous and non-aqueous dispersion media. Particle sizes were analyzed by dynamic light scattering (DLS) and analytical centrifugation (AC), and particle morphology by scanning electron microscopy (SEM). Physical stability was tested for sedimentation rate by AC, for dilution in biorelevant media by AC, and for particle growth of stored API nanosuspensions by SEM. The nanosuspensions were further characterized for their rheological behavior by flow curve, stress sweep and thixotropy. Finally, nanosuspensions' zeta-potential was characterized without sample dilution as a function of pH by electroacoustic spectrometry.
Results: The presented results show successful particle size reduction to below 250 nm of comp. A in aqueous and of comp. B in aqueous and non-aqueous dispersion media. The importance of the particle size measuring method (DLS and AC) for the actual result was evidenced for comp. B, which possessed either a rod-shaped or plate-shaped morphology depending on the dispersion media used. An optimal stabilizer formulation could be identified for comp. A showing a viscosity of below 10 mPas up to 1000 s^{-1} shear rate, no indication for flocculation, only marginal sedimentation, adequate zeta-potential of -20 mV, and robust particle size stability upon dilution in biorelevant media, e.g., SGF, FaSSIF and FeSSIF.

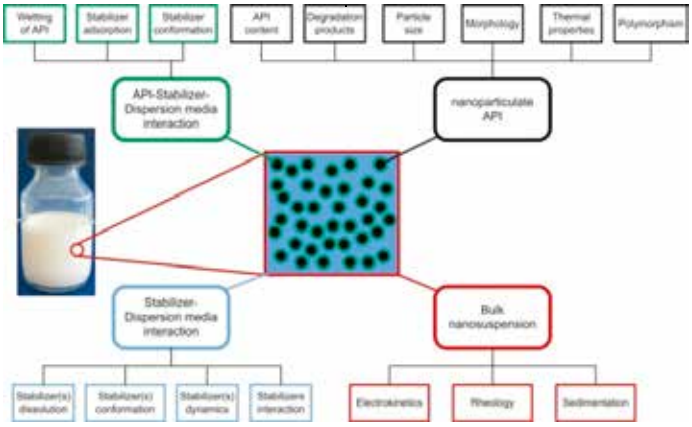

Fig. 1: Quality related and formulation related attributes of API nanosuspensions.

Conclusions: Directions for the formulation optimization can be immediately identified for quality (e.g. particle size), preparation (e.g. viscosity), storage (e.g. physical stability) and downstream processing (e.g. flocculation) attributes.

Keywords: Nanosuspension, formluation, characterization, stirred media milling.

P-10

Plants from West Papua Used Ethnomedically Against Malaria

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Introduction: West Papua remains one of the areas in Indonesia where malaria is still endemic. It has a high botanic diversity and many plants used ethnomedicinally to treat malaria. Yet these plants and uses have remained largely unstudied by modern science. The little work that has been done, mostly by local researchers, has not been easily available to the international scientific community.
Aims: To collect information on ethnomedicinally used plants from West Papua from primary and poorly accessible secondary sources.
Methods: We compiled information based on 5 locally published studies and interviewed local healers in the Sentani lake region.
Results: We compiled 29 plant species from 22 families, which were reported to be used to treat malaria. The part of the plants most frequently used were the leaves (48.3%), and the most common method for preparation was by decoction (48.3%). From these 29 plant species, extracts from just three have previously been tested *in vitro* and have shown antiplasmodial activity.
Conclusions: Ethnomedicinally used plants from West-Papua have been a neglected source of knowledge for the selection of plants for focused antiplasmodial screening. This study can serve as a source for such a selection.

Keywords: West Papua, ethnomedicine, malaria.

P-11

HPTLC Detection: An Appropriate Preparation of Spraying and Dipping Solutions

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Introduction, aims: In thin-layer chromatography, for the purpose of post-chromatographic derivatization, the specific derivatization reagent solution can be transferred onto the plate by spraying or dipping. This TLC step of detection, or derivatization, was studied with a view to elaborating a standardized approach. The focus was on the solvent used to prepare the derivatization solutions, and also on the concentrations of these reagent solutions.
Methods: For this purpose, qualitative results from both spraying and dipping experiments were compared. As herbal raw material, four flavonoid-containing drugs were used: ginkgo leaf, birch leaf, passion flower, and hawthorn leaf and flower. The specific derivatization reagent was natural product / macrogol 400; on the one hand dissolved in methanol, and on the other hand dissolved in ethyl acetate / dichloromethane [1]. The spraying as well as dipping procedures were carried out with appropriate automatic devices.
Results: Qualitative results showed that, in each case, derivatization reagents dissolved in ethyl acetate / dichloromethane showed concise zones, and weakly pronounced zones were also clearly visible. By contrast, dipping the plate into a derivatization solution containing methanol led partly to indistinct zones.
Conclusions: Thus, post-chromatographic derivatization by dipping required an adaptation of the original spraying reagent composition, i.e. specific solvent and concentration, to obtain comparable results between spraying and dipping. Such standardization of TLC steps may be helpful in the goal of harmonizing Ph. Eur. monographs.

Keywords: TLC, post-chromatographic derivatization, flavonoid-containg drugs.

Acknowledgments:
We thank SWISSMEDIC, Swiss Agency for Therapeutic Products, Pharmacopoeia division, for the financial support.

Reference:
[1] E. Reich, A. Schibli (2007): High-Performance Thin-Layer Chromatography for the Analysis of Medicinal Plants. Thieme, Stuttgart.

P-12

Pressurized Steam as Valuable Extraction Option to Produce TCM Decoction

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Introduction, aims: Decoction is one of the major forms of preparing drugs in traditional Chinese medicine (TCM). The traditional procedure consists of two boiling steps, e.g., 40 plus 20 min, of the drug in a suitable vessel [1]. However, other machines such as pressure cookers are coming more and more into discussion to prepare decoctions, e.g., for small-scale industry. The focus of this study was on the preparation of decoctions in pressurized steam (pressure cooker), especially on the resulting extraction yields and the time needed for preparation. These findings were compared to conventional decoctions prepared in a simple vessel on a hotplate, or with an electric brewing pot.
Methods: The experiment was carried out with the TCM drug aged tangerine peel (Chenpi, Citri reticulatae pericarpium), which contained hesperidin in the amount of 89.1 mg/g (=100%) in the herbal drug. Quantification was performed by HPLC analysis [2].
Results: The results of the pressurized steam process (small autoclave) showed a yield of hesperidin in the range of 6.2 -9.7%, with increasing yields according to the length of the extraction process (1 h–1 h 40 min, at 120 °C). By contrast, a conventional decoction (1 h soaking, and 40 + 20 min decoction) yielded only 3.4–3.9% of hesperidin, and a decoction in a brewing pot lasting 2 h, showed similarly low yields of hesperidin, namely 3.0–3.3%.
Conclusions: The present study showed that preparations of aged tangerine peel, Chenpi, extracted in pressurized steam contained a more than two-fold amount of hesperidin, compared to decoctions obtained by a conventional TCM procedure. Whether these findings could be generalized to other substances than hesperidin, or other drugs, will be the subject of further investigation.

Keywords: TCM, decoction, pressurized steam, hesperidin.

Acknowledgments:
We would like to thank Lian Chinaherb, Switzerland, for the supply of herbal drug material as well as SWISSMEDIC, Swiss Agency for Therapeutic Products, Pharmacopoeia division, for their financial support.

References:
[1] EDQM (2010): Preparation of drugs for traditional Chinese medicines, In: Pharmeuropa 22.2
[2] EDQM (2007): Mandarin epicarp and mesocarp (draft monograph), In: Pharmeuropa 19.1

P-13

Screening of South African Medicinal Plants and HPLC Based Profiling for the Identification of Leads with Antiprotozoal Activities

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Introduction: Diseases caused by protozoa are widespread in Southern Africa and there is a long tradition of using herbal remedies for their treatment [1].
Aims: To screen plants traditionally used to treat protozoal infections against the parasites *Plasmodium falciparum, Trypanosoma brucei rhodesiense, Trypanosoma cruzi* and *Leishmania donovani* and to identify their active constituents by HPLC based activity profiling.
Methods: 107 plants were screened. To identify antiprotozoal peaks in active extracts, 350 µg were fractionated by HPLC into 32 one-minute fractions in a fully automated 96 well microfractionation scheme [2], and microfractions were tested. HPLC hyphenated methods (MS, UV, ELSD, HRMS and offline LC-NMR) helped to identify active substances online.
Results: From the screened library, 102 (34%) exhibited more than 50% growth inhibition of one of the parasites at the concentration of 9.7 µg/mL and were thus active. *P. falciparum* against which 72 plant extracts (24%) showed activity was the most susceptible parasite, followed by *L. donovani* (49, 16%) and *T. brucei rhodesiense* (36, 12%), with *T. cruzi* (0) being the least susceptible.
Conclusions: Twenty plants (77%) were selected for further investigation based on activity and specificity criteria as well as on chemotaxonomic considerations. Screening results as well as selected examples of activity profiling will be shown.

Keywords: Anti-parasitic diseases, HPLC profiling, South African plants.

References:
[1] B. Pylla, J Ethnopharmacol 2008; 119: 438-54.
[2] M. Adams et al. Nat Prod Comm 2009; 10: 1377-8

P-14

Metabolomics Reveals Acute Resistance of the Rat Sebaceous Gland to Gamma-Radiation

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Aims: The aim of this work was to use metabolomics to evaluate sebum as a source of biomarkers for gamma-radiation exposure in the rat, and potentially in man. Proof of concept of radiation metabolomics was previously demonstrated in both mouse and rat urine, from the radiation dose- and time-dependent excretion of a set of urinary biomarkers.
Methods: Rats were gamma-irradiated (3 Gy) or sham irradiated and groups of rats were euthanized at 1 h or 24 h post-irradiation. Sebum was collected by multiple washings of the carcasses with acetone. Nonpolar lipids were extracted, methylated, separated and quantitated using gas chromatography-mass spectrometry (GC/MS). Metabolomic analysis of the GC/MS data was performed using principal component analysis (PCA) and both orthogonal projection to latent structures-discriminant analysis (OPLS-DA) and random forests machine learning algorithm (RF).
Results: Irradiation did not alter sebum production. Thirty-five lipids were identified in rat sebum, 29 fatty acids, 5 fatty aldehydes, and cholesterol. Metabolomics showed that 3 fatty acids, palmitic, 2-hydroxypalmitic, and stearic acids were potential biomarkers. Sebaceous palmitic acid was marginally statistically significantly elevated (7.5–8.4%) at 24-h post-irradiation.
Conclusions: Rat sebaceous gland appears refractory to 3 Gy gamma-irradiation. Unfortunately, collection of sebum shortly after gamma-irradiation is unlikely to form the basis of high-throughput noninvasive radiation biodosimetry in man.

Keywords: Metabolomics, sebum, gamma-radiation, fatty acids.

P-15

Adherence to Glivec®: Pattern of Refill in Community Pharmacies

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Introduction and Aims: Few published studies have reported low adherence rates in cancer patients. The objectives of the study were to assess refill adherence to imatinib (Glivec®) in ambulatory patients suffering from chronic myeloid leukemia, to identify their adherence behaviour and to evaluate how community pharmacists address adherence.
Methods: Retrospective observational study in community pharmacies in the region of Basel followed by a prospective study with Glivec® patients. Pharmacists were enrolled if they had Glivec® patients with refill claims and asked to recruit patients by phone or during their next visit. A trained master student conducted semi-structured interviews with pharmacists (during a personal visit) and with patients (by phone call). Patients filled out post-mailed questionnaires about beliefs about medicines (BMQ), adherence (MARS, MMAS-8) and literacy/numeracy (NVS). Main Outcome Measures: Prescription refill data allow to calculate the medication availability or possession ratio (MPR; percentage of days supply obtained during the period of refill intervals) and the continuous medication gaps (CMG; percentage of treatment gaps observed during the pe-

riod of refill intervals). High scores obtained with the questionnaires indicate strong beliefs in concerns about or necessity of the medication (BMQ), high self-reported adherence (MARS, MMAS-8) and high literacy/numeracy (NVS).
Results: Of the 112 pharmacies phoned in March 2009, 36 had at least one Glivec® patient. 12 refused to join the study. The 24 remaining pharmacists (mean age: 47.8 ± 11 y. (range: 25–66 y.), 33% men) recruited 19 patients (mean age: 66.1±11.8 y. (range: 48–84 y.), 57.9% men). All pharmacists stored at least Dosette® and none shelved Glivec®. One pharmacist would never address adherence with clients. The patients had on average 27.7 refills (range 5–82 refills) during a mean of 2.5 y. (range 4 months–6.4 y.). All patients but 2 got enough information, mostly from the hospital (12/19) but also from the Internet (6/19). All patients kept their medication in a visible place, either in the kitchen (68.4%), in the bathroom (21.0%) or in the living room (10.5%). Refill adherence (MPR: 95.7% ± 11.1 (range: 70–113%, max. value 100) and self-reported adherence (MMAS-8: 7.2 ± 1.2, max. value 8; MARS: 24.3 ± 1.2, max. value 25) were very high. Gaps in supply reached a mean of 11.7% ± 8.0 (range: 3.8–30.2%). The Pearson correlation analysis showed an association between CMG and total supply ($r = -0.48$; $p < 0.05$), CMG and MARS ($r = -0.54$, $p < 0.05$) and MPR and MARS ($r = 0.53$; $p < 0.05$). Patients with few refills tended to have gaps, to be older and to declare non adherence.
Conclusion: This pilot study revealed a reserved attitude of community pharmacists toward addressing adherence with their clients. MARS questionnaire may detect non adherent behaviour, which occurs mostly at the beginning of a Glivec® therapy.

Keyword: Adherence, pharmacotherapy, Glivec®, imatinib, community pharmacists.

P-16

Misleading Score-Lines on Tablets: Facilitated Intake or Fractional Dosing?

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Introduction: The presence of a score-line on a tablet is broadly interpreted as the tacit permission to fractionate the dose. If so, the fragments should comply with the content or mass uniformity requirements of the European Pharmacopoeia. For health professionals, information on divisibility is expected to be listed in the Summary of Product Characteristics (SPC), the Package Leaflets (PL) or in Hospital Drug Formularies.
Aims: To screen the SPCs and the PLs of "scored" tablets for information on divisibility and fractional dosing.
Methods: We selected the tablets specified as "scored" in the Swiss Compendium Online by 23rd October 2008 and in the current Drug Formulary of the University Hospital Basel dated 8th February 2008. Missing data was obtained from the marketing authorization holders.
Results: The Compendium contained 698 different scored tablets whose SPCs explicitly mentioned the possibility of fractional dosing for 43.8% of them. The Hospital Drug Formulary indexed 188 items as scored tablets. The corresponding SPCs mentioned fractional dosing for 107/188 (56.9%) and a sentence prohibiting it for 5/188 (2.7%). A fractional dosing according to manufacturers' answers was permitted for 49 (26%) of the remaining tablets and was prohibited for 19 (10.1%) of them. Lack of dosage uniformity within the tablet fragments or presence of "historic decorative" score-lines were the evoked reasons for the interdiction.
Conclusions: For the majority of scored tablets, an explicit statement on fractional dosing is not available in the official drug information. Sentences noting interdiction in the SPCs are easily overlooked. The simple notation "divisible" in the Hospital Drug Formulary is misleading into a prohibited fractional dosing for 12.8% of the indexed scored-tablets.

Keywords: Scored tablets, score line, fractional dosing, splitting, divisibility.

P-17

Comparative Study of the Critical Points of Hydrophilic Matrices Based on Hypromellose K4M CR

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Introduction, aims: The percolation theory is a statistical theory able to study chaotic or disordered systems that has been applied in the pharmaceutical field since 1987. Through the application of this theory, the design of controlled release hydrophilic matrices has been improved [1–3]. The aim of the present paper is to estimate the percolation thresholds, the most important concept of the percolation theory, which characterise the release behaviour of controlled release hydrophilic matrices and to evaluate the role of the initial porosity of the system. Formulations studied here contained carbamazepine as a poor water soluble model drug and one hydrophilic matrix forming polymer METHOCEL™ K4M CR, and matrices with different porosities were manufactured. Fillers and lubricant mixtures have also been included.
Methods: Preparation of matrix tablets: Initially, all materials, with the exception of magnesium stearate and colloidal silicon dioxide, were blended for 10 min in a Turbula mixer. Magnesium stearate and colloidal silicon dioxide were added and blended for an additional period of 5 min. The matrix tablets (600 mg, 12-mm diameter) were prepared by direct compression, using a standard eccentric tablet machine (Bonals A-300). The polymer concentrations were 15, 20, 25, and 30% w/w for the 15 assayed lots. Dissolution testing of the matrix tablets was subjected to a modified dissolution assay. More stressed hydrodynamic conditions were used in comparison to the standard assay, in an attempt to a faster determination of the critical points of the system: 900 mL of distilled water at 37 ± 0.5°C, USP II paddle method, rotational speed 150 r.p.m. Samples were withdrawn at 0.25, 0.5, 1, 1.5, 2, and 3 h. The percent of carbamazepine released was measured via UV spectrophotometry (Hitachi U-2000) at a wavelength of 284 nm. The assay was performed in three replicates. Evaluation of the release mechanism: Release data analyses were performed using Higuchi (1963), Korsmeyer et al. (1983) and Peppas and Sahlin (1989) equations and SYSTAT program. Linear (Higuchi) and non-linear least squares fitting methods were used to determine the optimum values for the parameters included in each equation [1, 4].
Results and conclusions:
Critical porosities ranges found for HPMC K4M CR:
- Minimum porosity range: 11.7–15.4% v/v.
- Medium porosity range: 13.6–18% v/v
- Maximum porosity range: 15.1–19,8% v/v.

Although different porosities and compression forces have been employed, the release profiles were compatible with a common critical point around 15% v/v of HPMC K4M CR, indicating the robustness of this parameter. The studied properties show a critical behaviour as a function of the volumetric fraction of the components, as percolation theory predicts, which can be attributed to the polymer percolation threshold.

Keywords: Percolation theory, hydrophilic matrix, HPMC K4M CR, critical points.

References:
[1] A. Miranda, M. Millán, and I. Caraballo, Int J Pharm 2006; 311: 75-81.
[2] A. Miranda, M. Millán, and I. Caraballo. J Pharm Sci 2007; 96: 2745-2756.
[3] I. Fuertes, A. Miranda, M. Millán, and I. Caraballo. Eur J Pharm Biopharm 2006; 64: 336-342.
[4] T. Gonçalves, 2007. Percolation threshold in hydropilic matrix obtained by ultrasonic assisted compression and industrial formulation. PhD Thesis. University of Seville, Spain.

P-18

Biochemical Characterization of the Thymidine Kinase from *Leishmania donovani*

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Introduction: Leishmaniasis is a vector-borne parasite disease transmitted to human by protozoan of the genus *Leishmania* via the bites of the sandflies. The first line drugs employed to cure leishmaniasis are associated with side effects and toxicity. This considerably limits their use. Moreover, parasites have developed resistance towards the drugs. Therefore, the identification and validation of new targets are crucial to develop new effective treatments. A recent study on *Leishmania major* and *Leishmania donovani,* including a genetic validation indicated that *Leishmania* thymidine kinase could play a role in the virulence of the parasite [1-2], thus could be seen as putative target for a therapeutic intervention using inhibitors.
Aims: The aim of the work is to assess the *Leishmania donovani* thymidine kinase, *Ld*TK1, as a therapeutic target against leishmaniasis. *Ld*TK1 belongs to the type II thymidine kinase and shares 51% identity of sequence with the human cytosolic TK1 (hTK1) [3-4]. This guarantees that the fold is conserved but that there are enough differences in the binding site for achieving selectivity with a compound.
Methods: To achieve one part of the work, we used biochemical methods to characterize at the molecular level *Ld*TK1, including its recombinant expression of the enzyme, functional characterization (spectrophotometric activity assay [5], radiometric assay with the DEAE-cellulose method [6], screening of compounds for their inhibition potential (HPLC assay [7]).
Results: The *ldtk1* gene was cloned and the *Ld*TK1 overproduced and purified in its active form. The phosphorylation assays showed that the recombinant *Ld*TK1 is able to phosphorylates dT (3'-deoxythymidine), BrdU (5-bromo-deoxy-uridine), AZT (3'-azido-3'-deoxythymidine), FdU (5-fluoro-2'-deoxyuridine). *Ld*TK1 is inhibited by dTTP. *Ld*TK1 doesn't phosphorylate and is not inhibited by the acyclic analogues of deoxyguanosine. The determination of the kinetics parameters is currently underway.
Conclusions: The outcome of the biochemical characterization will serve as proof of principle for an *Ld*TK1 based inhibitory therapeutic intervention for leishmaniasis and contribute to better understand the biology of the *Leishmania* parasite.

Keywords: *Leishmania donovani,* thymidine kinase, therapeutic target.

References:
[1] Thiel et al. Mol Biochem Parasitol 2008; 158: 152-62.
[2] Bente et al. Proteomics 2003; 3: 1811-29.
[3] Birringer et al. FEBS Lett 2005; 579: 1376-82.
[4] Birringer, et al. Protein Expr Purif 2006; 30: 30.
[5] Schelling et al. Anal Biochem 2001; 295:82-7
[6] Gerber et al., Biochem Biophys Res Commun 1996; 225:263-67
[7] Pilger et al., J Biol Chem 1999; 274, 31967-73

P-19

Preparation of Respirable Protein Powder Using a Nano Spray Dryer

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Introduction: Spray drying is a common method to dry biopharmaceuticals due to the mild process conditions. Furthermore, it allows producing particles of controlled size and shape. This is important for the production of respirable powders, which could in the future be used as an alternative to the parenteral delivery of proteins.
Aim: The aim of this study was to evaluate the Nano Spray Dryer B-90 (BÜCHI Labortechnik AG, Flawil, Switzerland) with regard to the drying of proteins. The objective was to produce particles of respirable size (1–5 µm) with full enzyme activity at maximized yield. Furthermore, the influence of formulation and process parameters on particle morphology and storage stability was examined.
Methods: β-Galactosidase was chosen as a model protein and spray-dried together with trehalose as a stabilizer. A 3^3 full factorial design was applied to determine the influences of the inlet temperature (T_{in}), the spray cap size (C) and the ethanol concentration in the spray solution (E). The residual enzyme activity was determined colorimetrically and the particle size was measured by means of laser diffractometry. Particle morphology was studied using scanning electron microscopy. For the stability test, the samples were stored at an elevated temperature.
Results: The inlet temperature as well as the interaction of inlet temperature and spray cap size had a significant influence on the residual enzyme activity. Full enzyme activity could be retained with the optimized formulation and process settings. The median particle size was significantly affected by the ethanol content and the hole size of the spray cap membrane. Higher ethanol contents and smaller spray cap sizes resulted in smaller particles. Contrary to the other spray cap sizes, the smallest spray cap led to a monodisperse size distribution, which resulted in a greater fraction of particles of respirable size (52%). Yields of up to 94% were achieved for 500-mg powder amounts. Higher yields were found at lower inlet temperatures, higher ethanol contents and with smaller spray cap sizes. The particle morphology was generally spherical. Particles with a smooth surface and shrivelled particles were found, depending on the cap size. There were remarkable differences in storage stability between the different samples (0%–43% activity loss). Samples produced with a larger cap size and without ethanol in the spray solution showed better storage stability.
Conclusions: This study showed, that β-galactosidase could be spray dried without activity loss using the Nano Spray Dryer B-90 with the optimized process setting (T_{in}: 80 °C, C: 4 µm, E: 0%). Furthermore, this setting allowed producing particles of respirable size and it resulted in high yields (approximately 90%).

Keywords: Spray drying, nano spray dryer, inhalation, β-galactosidase, trehalose.

P-20

Investigation of Variability of Primary Materials on the Intrinsic Dissolution Behavior of Carbamazepine

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Introduction: Carbamazepine (CBZ) formulations show a high risk for clinical failures due to polymorphism and morphologic variations of drug raw material. These two attributes are critical parameters concerning dissolution behavior.
Aims: The aim of this study was to characterize CBZ polymorphic form III (P-monoclinic) powders provided from four different suppliers. Deviations in dissolution behavior were investigated using disc intrinsic dissolution rate (DIDR) measurement. In order to eliminate morphologic variations among the CBZ samples recrystallization of the bulk materials from ethanol in presence of polyvinylpyrrolidone (PVP) was done. The influence of micro-crystalline cellulose (MCC) on the dissolution behavior of the CBZ samples was studied using simple formulations.
Methods: Polymorphic form and morphology of CBZ bulk material were characterized by sieve analysis, differential scanning calorimetry, powder X-ray diffractometry, scanning electron microscopy, and light microscopy. Flat-faced compacts with a surface area of 0.95 cm^2 were prepared by Zwick material tester. Tablets consisted of pure CBZ, recrystallized CBZ, and CBZ-MCC powder mixtures (90, 70, 50, and 30% drug load), respectively. DIDR measurement and dissolution tests were conducted using a closed system of SotaxAT7smart with modified rotating baskets and UV spectroscopy at 285 nm. Analyses were done in 500 mL water at 37 °C and 100 rpm for 2 h. To evaluate DIDR profiles and dissolution data statistical analysis (ANOVA and T-student test) and mathematical models zero order, first order, Korsmeyer-Peppas, Power Law, and Weibull were applied.
Results: DIDR profiles of the pure CBZ samples differed significantly ($p<0.001$). Deviations in dissolution behavior were reduced among recrystallized CBZ samples ($p<0.01$). In powder mixtures the variability of CBZ gave also different dissolution profiles, except for 50% CBZ loading ($p>0.05$). DIDR and dissolution profiles fit the applied mathematical models ($R^2 \geq 0.99$). Drug release mechanism was mainly classified as non-Fickian diffusion.
Conclusions: Recrystallization minimized differences among the CBZ samples, however, drug history was not reset. MCC influenced the dissolution behavior of CBZ, but variations among the bulk materials persisted. It is suggested to test further simple CBZ formulations and to perform recrystallization experiments of the bulk materials under various conditions.

Keywords: Polymorphism, morphology, intrinsic dissolution, recrystallization.

P-21

In Silico Toxicology Predictions Via Pharmacophore-Based Virtual Screening: The Discovery of the UV-Filter Benzophenone-1 as Inhibitor of Testosterone Synthesis

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Introduction: The prevalence of male reproductive disorders and testicular cancer is steadily increasing. Because the exposure to chemicals disrupting natural hormone action has been associated with these diseases, it is important to identify endocrine disrupting chemicals (EDCs) and their targets of action.
Methods: In this study, a 3D structural database that can be applied for virtual screening approaches to facilitate the identification of EDCs was constructed. As a first application scenario, the database was screened using pharmacophore models of inhibitors of 17β-hydroxysteroid dehydrogenase type 3 (17β-HSD3), which catalyzes the last step of testosterone synthesis in testicular Leydig cells and plays an essential role during male sexual development.
Results: Among other chemicals, benzophenone (BP) UV-filters were predicted as potential 17β-HSD3 inhibitors. Biological analyses revealed (2,4-dihydroxyphenyl)-phenylmethanone (also known as benzophenone-1, BP-1) as an inhibitor of human 17β-HSD3 (IC_{50} 1.05 μM). BP-1 also efficiently blocked conversion of androstenedione to testosterone by mouse and rat 17β-HSD3 in whole-organ enzyme assays. Moreover, BP-1 antagonized the testosterone-dependent activation of androgen receptors (IC_{50} 5.7 μM), suggesting synergistic anti-androgenic effects of BP-1 by preventing testosterone formation and blocking receptor activation.
Conclusions: According to these results, virtual screening of environmental chemical databases can facilitate the identification of compounds interfering with hormone action.

Keywords: UV-filter, inhibitor, pharmacophore, androgen, 17β-hydroxysteroid dehydrogenase.

P-22

Combination of Pharmacological Biomarkers and Compliance Monitoring to Detect Contributing Factors to Drug Resistance – A Study Design

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Introduction: The term "pharmacological biomarker" (Medical Subject Heading (MeSH) in the PubMed Database since 2008) applies to biological markers for the evaluation and improvement of drug efficacy and safety and may represent the only early indicator of drug effect in long-term therapy. In this context, drug resistance can be defined as an inadequate change of a specific biomarker under prescribed therapy, irrespective of the contributing factors. A focused analysis can disclose non-compliance, co-morbidities, drug-drug interactions, lifestyle factors and genetic polymorphisms, which are potential contributors to drug resistance. We adopted this model in a comprehensive study design to investigate the phenomenon of antiplatelet resistance with aspirin and clopidogrel.
Aims: (1) To develop a model for the investigation of drug resistance and (2) to adapt the model to the phenomenon of antiplatelet drug resistance.
Methods: Original research articles and reviews on drug resistance were identified in the PubMed database and were analyzed regarding their definition of drug resistance. Special attention was given to antiplatelet resistance. Experts in the field of antiplatelet therapy

(1 hemostaseologist and 1 cardiologist) were interviewed to finalize a consistent model of drug resistance.
Results: In our model, drug resistance is the resulting condition of potential contributing factors with clinical, cellular and genetic background. Clinical relevance of the condition is given by the established association of the pharmacological biomarker with specific clinical endpoints. The study design integrates electronic monitoring of multidrug compliance and biomarker measurements in a sequential approach. Repeated measurements of the pharmacological biomarker before and after electronically observed therapy will permit to separate non compliance from other contributors to resistance. The background of persistent antiplatelet resistance after exclusion of non compliance will be assessed by the focused analysis of potential contributing factors.
Conclusions: (1) The model developed for the investigation of drug resistance is adaptable to the specific problem of antiplatelet resistance. (2) The combination of a full compliance monitoring period with pharmacological biomarker measurements allows to figure out the impact of the involved factors and can help to uncover contributing factors both in research and in clinical practice.

Keywords: Drug resistance, compliance, electronic monitoring, pharmacological biomarker.

P-23

Malaria in the Renaissance: Remedies from European Herbals from the 16th to 18th Century

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Introduction: From antiquity up into the 20th century *Plasmodium vivax* and *P. malariae* malaria were widespread in Central Europe. Hundreds of different remedies against malaria can be found in herbals from the Renaissance.
Aims: To document and discuss old remedies described in German language, 16th and 17th century herbals in the viewpoint of modern science.
Methods: Nine of the most important European herbals of the 16th,17th and 18th century including Bock (1577), Fuchs (1543), Matthiolus (1590), Lonicerus (1560 & 1770), Brunfels (1532), Zwinger (1696), and Tabernaemontanus (1591 & 1678) were searched for terms related to malaria, and documented plants and recipes described for its treatment. Recent pharmacological data was found less than 5% of them.
Results: Three hundred and fourteen plants were identified in the herbals for this indication. Recent pharmacological data was found less than 5% of them.
Conclusion: European herbals may be a valuable source of information for the selection of plants for focussed antiplasmodial screening programmes, but have received only little scientific attention in the past.

Keywords: European herbals, malaria, renaissance, *Plasmodium vivax, Plasmodium malariae.*

P-24

Quantification and Profiling of Endogenous Steroids by UHPLC-QTOF-MSE

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Introduction and aims: Anabolic androgenic steroids (AAS) are mainly excreted in human urine as phase II metabolites (glucuronides and sulfates). Elevated levels of steroid metabolites in urine may result from an illegal intake of these molecules. The determination of testosterone and relatives are commonly performed by GC/MS after hydrolysis of the conjugated part and derivatization. The World Anti-Doping Agency (WADA) has determined criteria to consider testosterone abuse, such as the testosterone glucuronide to epitestosterone glucuronide ratio (T/E). A ratio ≥ 4 is considered as suspicious of testosterone misuse. However, the information, provided by the phase II metabolism, is partially lost during the hydrolysis step. Therefore, direct quantification of intact steroid conjugates is still challenging. A method has been developed herein to detect glucuronide and sulfate conjugates simultaneously with satisfying sensitivity and selectivity.
Methods: Ultra-high-pressure liquid chromatography (UHPLC) coupled to hybrid quadrupole time-of-flight (QTOF) mass spectrometry was selected for this purpose. UHPLC offers high chromatographic performance by using columns packed with small particles (i.e. sub-2µm), allowing high peak capacity within reasonable analysis time, thus providing baseline separation between pairs of isomers. Furthermore, the QTOF mass analyzer allows exact mass determination. After a sample preparation by SPE on HLB cartridges, a highly selective chromatographic separation was performed within a single gradient elution of 36 min (including re-equilibration time) in the negative ESI mode and 2 functions were acquired simultaneously in the MS^E mode.
Results: This approach allows the quantification of the investigated metabolites by assessing the molecular ion obtained in the first function at low collision energy (5 eV) and a rich fragmentation pattern helping the identification of testosterone relatives is provided in the second function acquired at high collision energy (ramp from 5 to 70 eV). The method was validated and found suitable for conjugated steroids measurement in human urine. It was thus applied to samples of a clinical study performed with one group of healthy volunteers having taken testosterone compared to a placebo group.
Conclusions: The wealth of information provided by the exact mass determination and the fragmentation pattern allows the identification of compounds and open the way to a broader steroid profiling including an extensive monitoring of endogenous steroids (steroidomics).

Keywords: Anabolic androgenic steroids, phase II metabolism, ultra-high-pressure liquid chromatography, quadrupole time-of-flight, quantification.

P-25

Cross-Linkable Polymers for Nanocrystal Stabilization

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Introduction: Nanoparticulate drug delivery systems are currently used to overcome critical challenges associated with classic dosage forms. Formulating hydrophobic drugs as "nanocrystals" is very interesting because of the use of little excipient with respect to drug, thereby reducing the risk of toxicity from this component.
Aims: Nanoparticles of paclitaxel (model drug) with target sizes of about 170 nm were prepared by wet milling and stabilized by tailored copolymeric surfactant composed of methoxy-poly(ethylene glycol)-*b*-poly(ε-caprolactone) (mPEG-*b*-PCL), which bear either alkynyl or azido groups incorporated in the PCL block. These functional groups were then reacted in a copper(I)-catalyzed 1,3-dipolar cycloaddition reaction. It is our hypothesis that this cross-linked polymer coating will help to stabilize the drug nanocrystal and permit control over its rate of dissolution.
Methods: Functional monomers (Fig. 1: **1, 2**) were synthesized and copolymerized with ε-capro-lactone (CL) from mPEG via cationic ring opening polymerization (CROP). Wet milling of the drug was performed in a cylindrical glass vessel containing an aqueous suspension of the dissolved polymer **(3, 4)** and solid drug with 0.3-mm zirconium oxide beads, which was rotated at 220 rpm for 48 h. Catalyst ($CuSO_4$ / ascorbic acid) was added to this colloidal dispersion and reacted for 20 min at room temperature. ^{1}H NMR and FTIR spectroscopy were used to evaluate the process of this reaction.

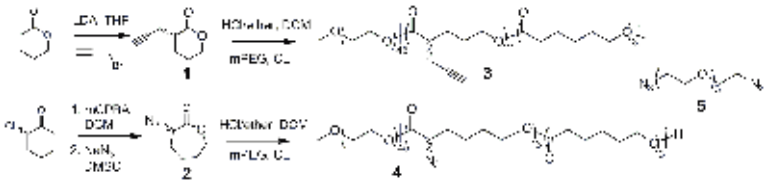

Fig. 1: Synthesized monomers (α-propargyl-δ-valerolactone 1, α-azido-ε-caprolactone 2) and polymers (3,4); 5 was commercially obtained.

Results: Conditions for click chemistry were optimized in solution using **3** and **5**. Reaction was confirmed by the disappearance of characteristic bonds from the alkyne (strong ≡C–H stretch at 3270 cm^{-1}) and azide (band at 2100 cm^{-1}) in FTIR spectra and appearance of a new peak for the triazole (8.10 ppm) in ^{1}H NMR spectra. The conditions employed above were exploited for the reaction in the presence of nanocrystals with **3** and **4.** Complete reaction of the alkynyl groups (peak disappearance in FTIR) and formation of a triazole (^{1}H NMR) were again observed. From this result it was concluded that the functional groups remained active, even when adsorbed to the crystal.
Conclusions: In this work, functional copolymers were successfully prepared by transition metal-free CROP. Reaction of the alkynyl groups with azido compounds was successful in the presence of nanocrystals. Successful cross-linking of the polymers around the drug nanoparticle may serve as a powerful tool to control dissolution rate and thereby circulation time in the body, which could enhance tumoral uptake *via* the EPR effect.

Keywords: Nanomedicine, wet milling, click-chemistry, paclitaxel, nanocrystals.

P-26

Proline-Specific Endopeptidases – *In Vitro* Stability and Activity

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Introduction: Celiac disease (CD) is one of the most prevalent (0.5–1%) gut-associated illnesses worldwide. Wheat proteins (*i.e.,* gluten) induce a T cell-mediated inflammation of the intestine in genetically predisposed individuals. Currently, lifelong exclusion of wheat gluten and related cereals (*i.e.,* barley and rye) is the only available treatment for CD. Proline-specific endopeptidases (PEP) can efficiently cleave gluten peptides and thus minimize the toxicity for CD patients. However, their oral delivery remains challenging due to pH changes and digestive enzymes along the gastrointestinal tract.
Aims: The present work systematically evaluates the stability of known PEPs in artificial gastrointestinal fluids and studies the route by which these enzymes are inactivated *in vitro.* Additionally, the proteolytic activity of PEPs using known immunotoxic peptides and gluten was determined. This study incorporated PEPs from *Flavobacterium meningosepticum* (FM), *Myxococcus xanthus* (MX), and *Sphingomonas capsulate* (SC).
Methods: The stability of PEPs was evaluated by incubating individual enzymes at pH 1.2, 4.5, and 6.8, as well as in simulated gastric or intestinal fluid USP. Residual activity of samples was analyzed using the dipeptic model substrate *Z*-Gly-Pro-*p*NA. The principal routes of inactivation were explored by means of circular dichroism spectroscopy (CDS) and sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS PAGE). For activity determination, the toxic peptide PQPQLPYPQPQLP (13mer) or gluten predigested with pepsin-trypsin-chymotrypsin (PTC-gluten) was incubated with PEPs (100:1 *w/w*) at 37°C and pH 4.5 or 7.0. Samples were analyzed using liquid chromatography-mass spectrometry (LC/MS) and the abundance of peptides of interest was normalized to a non-PEP treated sample.
Results: Incubation of individual PEPs under artificial gastrointestinal conditions lead to rapid inactivation of the enzymes. In an environment simulating the stomach, none of the PEPs retains more than 25% of its initial activity after 10 min. Low pH and the presence of pepsin rapidly denatures the PEPs. Similar results were obtained under artificial intestinal conditions. More specifically, the presence of pancreatic proteases impaired PEP's stability (10% remaining activity after 20 min). CDS measurements revealed that incubation at low pH leads to a pronounced loss in α-helical structure. Additionally, PEPs are prone to lysis as determined by SDS-PAGE. PEPs were very active toward toxic gluten peptides. The 13mer was efficiently cleaved by all PEPs within 120 min at intestinal pH 7.0. Furthermore, PTC-gluten was detoxified by PEPs at stomach pH 4.5. Notably, FM PEP reduced the abundance of an exemplary gluten peptide within 30 min.
Conclusions: The results demonstrate the efficient cleavage properties of PEPs, but moderate stability of these enzymes when incubated under simulated gastrointestinal conditions. To overcome these drawbacks polymer modifications of PEPs are planned for upcoming studies.

Keywords: Proline-specific endopeptidases, celiac disease, gluten peptides.

P-27

Biological Assessment of Novel Glycyrrhetinic Acid Derivatives Acting as Selective Inhibitors for 11β-Hydroxysteroid Dehydrogenase Type 2

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Introduction: Intracellular availability of glucocorticoids and activation of glucocorticoid receptors (GR) is tightly regulated by 11β-hydroxysteroid dehydrogenase 1 (11β-HSD1) and 11β-HSD2. 11β-HSD2 catalyzes the conversion of active 11β-hydroxyglucocorticoids (cortisol, corticosterone) into inactive 11-ketoglucocorticoids (cortisone, 11-dehydrocorticosterone) and is expressed in kidney, colon, sweat and salivary glands but also in placenta, inflamed tissue and many tumors and cancer cell lines. Impaired local glucocorticoid metabolism has been associated with several disease states, and modulation of intracellular glucocorticoid availability is considered to be a promising strategy to treat glucocorticoid dependent diseases. 18β-glycyrrhetinic acid (GA), the biologically active triterpenoid of the roots and rhizomes of licorice *(Glycyrrhiza),* represents a well known but non-selective inhibitor of both 11β-HSD enzymes. However, for therapeutic applications selective inhibitors are needed. Therefore, the aim of the present study was the biological characterization of a set of novel and selective 11β-HSD2 inhibitors.
Methods: The activities of selected inhibitors were compared in assays using lysates and intact cells expressing recombinant human enzymes. Possible species-specific differences were considered by comparing inhibitory activities of the compounds on human and mouse 11β-HSD2. Further, the impact of the GA derivatives on 11β-HSD-dependent modulation of GR transactivation activity was assessed. In an attempt to understand the selectivity of the GA derivatives to inhibit 11β-HSD1 and 11β-HSD2, respectively, an 11β-HSD2 homology model based on structural information on the related 17β-HSD1 was generated and applied together with our recently constructed pharmacophore of 11β-HSD1.
Results: Structural modification of the functional groups of the triterpenoid backbone led to derivatives that selectively inhibited either 11β-HSD1 or 11β-HSD2. Several derivatives with modifications at the 3-hydroxyl and the carboxyl group of GA showed high selectivity for 11β-HSD2, with IC_{50} values in the nanomolar range. The data generated lead to a significant extension of the knowledge about structure activity relationship of GA derivatives as inhibitors of human 11β-HSDs.
Conclusions: The structural analyses provide an explanation for the differences in the selectivity and activity of the GA derivatives investigated. The most potent and selective 11β-HSD2 inhibitors should prove useful as mechanistic tools for further anti-inflammatory and anti-cancer *in vitro* and *in vivo* studies.

Keywords: 11β-Hydroxysteroid dehydrogenase, pharmacophore, inhibitor, glucocorticoid, glycyrrhetinic acid.

P-28

Discovery of 11β-Hydroxysteroid Dehydrogenase 1 Inhibiting Triterpenes from *Eriobotrya Japonica* by Bioactivity-Guided Isolation and Computational Approaches

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Introduction and aims: Elevated tissue-specific activation of glucocorticoids by 11β-hydroxysteroid dehydrogenase 1 (11β-HSD1) has been associated with metabolic disorders including obesity and type 2 diabetes. Recent findings provided evidence for beneficial effects of 11β-HSD1 inhibitors, making this enzyme a promising therapeutic target. In order to identify natural compounds inhibiting 11β-HSD1, we recently investigated different extracts of traditionally used anti-diabetic medicinal plants for potential anti-glucocorticoid effects. The extracts of the leaves of the anti-diabetic medicinal plant Loquat *(Eriobotrya japonica)* showed a dose-dependent inhibition of 11β-HSD1 and a preference to inhibit 11β-HSD1 versus 11β-HSD2. The present study aimed at the identification of 11β-HSD1 inhibiting compounds from *E. japonica*.
Methods: In the present study, the leaf extracts of *E. japonica* were phytochemically investigated, following hints from a pharmacophore-based virtual screening and a bioactivity-guided approach. Extracts and isolated compounds were tested for 11β-HSD1 and 11β-HSD2 inhibitory activities in cell lysates.
Results: Biological analyses revealed triterpenes from the ursane type as selective, low µmolar inhibitors of 11β-HSD1: corosolic acid (IC_{50} 0.8 µM), 3-epicorosolic acid methyl ester (IC_{50} 5.2 µM), 2-α-hydroxy-3-oxo-urs-12-en-28-oic acid (IC_{50} 17 µM), tormentic acid methyl ester (IC_{50} 9.4 µM), and ursolic acid (IC_{50} 1.9 µM). Intriguingly, a mixture of loquat constituents with moderate activities displayed a pronounced additive effect. By means of molecular modeling studies and the identification of the 11β-HSD1 inhibiting 11-keto-ursolic acid (IC_{50} 2.1 µM) and 3-acetyl-11-keto-ursolic acid (IC_{50} 1.3 µM), a structure-activity relationship was deduced for this group of pentacyclic triterpenes.
Conclusions: The mechanism of action elucidated in this study together with the previously determined pharmacological activities provides this class of compounds from Loquat with an astonishing multi-targeted anti-diabetic profile.

Keywords: 11β-hydroxysteroid dehydrogenase, pharmacophore, inhibitor, glucocorticoid.

P-29

Influence of the Clozapine Particle Size on the Release Behaviour of Matrix Pellets

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Introduction: The percolation theory, introduced into the pharmaceutical field by Leuenberger and co-workers has been extensively applied to the study of the release behaviour of inert and hydrophilic matrices facilitating the design and optimisation of numerous pharmaceutical formulations. Pellets formulations, as multiparticulate systems, offer a wide range of therapeutic and technological advantages such as drug absorption being independent from gastric emptying rate, reduced intra- and inter-individual variability of drug plasma levels, and very good flow properties.
Aims: The aim of this study is to investigate if it is possible to explain the release behaviour of clozapine matrix pellets applying the concepts of the percolation theory, previously used in the understanding of the release process of inert and hydrophilic matrix tablets.
Methods: Thirteen batches of pellets with different concentrations of clozapine/MCC/HPMC were prepared by extrusion (Pharmex 35T Gabler Machinenbau) -spheronisation (Sphaeromat SPH 250 MA Gabler Machinenbau) containing varying particle size of clozapine obtained by sieving. Release studies were performed in a USP rotating basket apparatus with 900 mL of pH 4 acetate buffer.
Results: It has been observed that the distance to the drug percolation threshold has a significant influence on the release rate. Batches very close to the drug percolation threshold, show a clear effect of the drug particle size in the release rate. This effect is however much less evident when distancing from the drug percolation threshold. This can be explained based on the increase in the percolation threshold caused by an increase in the particle size.
Conclusions: Despite the differences in the preparation techniques, the release behaviour of clozapine matrix pellets can be explained using similar concepts than the previously applied to tablets, based on percolation theory.

Keywords: Pellets, clozapine, HPMC, percolation threshold, particle size.

P-30

Phenolic Compounds from the Leaves of *Garcinia Preussii*

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Introduction: In the course of the study of medicinal plants from Cameroon, the leaves of *Garcinia preussii* Engl (Clusiaceae) were investigated. The plants of this genus are mostly used for their antimicrobial properties. Chewing sticks are used for dental hygiene by most people in western Africa. This genus is well known as a good source of biological active metabolites such as xanthones, benzophenones and biflavonoids. Some of previously isolated xanthones exhibited significant acetylcholinesterase inhibitory activity, as well as antioxidant, and antimicrobial effects.
Methods: The methanolic extract of the fresh leaves of *G. preussii* was filtered on polyamide cartridge and submitted to successive chromatographic separations using a combination of normal phase and Sephadex LH-20 chromatography with various solvent systems. The structures of isolated compounds were elucidated by spectroscopic and chemical methods including 1D- and 2D-NMR experiments and MS analysis.
Results: Compounds such as xanthone glycoside, flavonoids and steroids were identified. To our knowledge, flavonoid *O*-glycosides are reported for the first time in the genus *Garcinia*. The antioxidant activity of these compounds was also evaluated.

Keywords: *Garcinia,* xanthone glycoside, flavonoids.

P-31

New Flavone 8-C-Glycosides from *Haberlea Rhodopensis* Friv.

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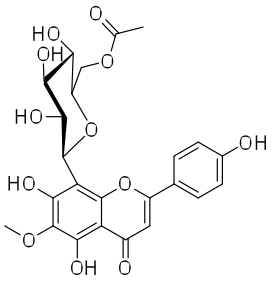
Introduction: *Haberlea rhodopensis* Friv. (Gesneriaceae) is a perennial resurrection plant native of the Balkans. As a poikilohydric organism, *H. rhodopensis* is dessication tolerant [1]. Its behavior under dehydration and rehydration has been the subject of several photosynthetic and metabolic studies. On the other hand, information on its secondary metabolites remains scarce.
Aims: The major chemical constituents of methanolic extract of *H. rhodopensis* leaves were investigated by using HPLC-UV, HPLC-ESI-TOF-MS and NMR techniques.
Methods: The n-butanol portion of the MeOH extract of leaves of *H. rhodopensis* was subjected to RP-18 by using preparative and semi-preparative HPLC systems. The HPLC analysis of the n-butanol portion revealed the presence of five major peaks.
Results: The fractionation of a methanolic extract of the leaves of *H. rhodopensis* by a combination of liquid/liquid solvent extraction, preparative and semi-preparative HPLC on RP-18 yielded two new flavones C-glycosides, namely hispidulin-8-C-(6-O-acetyl)-*ß*-D-galactopyranoside **(1),** and hispidulin-8-C-[6-O-acetyl-2-O-(4-hydroxy-3,5-dimethoxybenzoyl)]*ß*-D-galactopyranoside **(2),** along with two known phenolic glycosides, i.e. myconoside and pauciflosides.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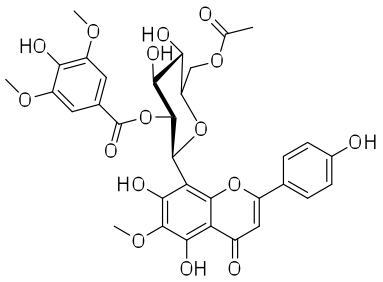
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Conclusions: The structures of **1** and **2** were established by extensive spectroscopic measurements including 1D- and 2D-NMR (COSY, HSQC, HMBC) and HR-ESIMS.

Keywords: *Haberlea rhodopensis,* Gesneriaceae, flavones C-glycosides, profiling.

Reference:
[1] Y.K. Markovska, T.D.Tsonev, G.P.Kimenov, A.A.Tutekova, J Plant Physiol 1994; 144: 100.

P-32

Antiplasmodial Compounds from Roots of *Salvia Sahendica*

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Introduction: Iran has a long-standing medical tradition and vast traditional knowledge of plant remedies. Plants used to treat ma-

laria or cancer or microbial diseases in different areas from Iran were selected for this investigation. Selected plants were collected and further evaluated for their antiplasmodial activity.
Aims: Medicinal plants belonging to the families Lamiaceae and Asteraceae used in different areas of Iran were selected and submitted to a screening for antiplasmodial activity. A total of 102 plant extracts were tested against *Plasmodium falciparum* K_1 *strain.*
Methods: The antiplasmodial compounds in *Salvia sahendica* (Lamiaceae) were tracked by HPLC-based activity profiling using a recently established protocol. Active compounds were subsequently isolated by preparative chromatography, and their structures elucidated by 1D- and 2D-NMR (COSY, HSQC, and HMBC) in combination with hyphenated techniques (HPLC-PDA and HPLC-HR TOF-MS).
Results: One of the most active samples was a hexane extract of the roots of *Salvia sahendica,* an endemic plant to Iran, which showed >70% inhibition at a test concentration of 0.85 µg/mL. The activity profile obtained was matched with the HPLC trace.
Conclusions: Three diterpenes, i.e. 12-deoxy-salvipisone, sahandinone, and ferruginol, were identified as compounds responsible for the activity.

Keywords: *Salvia sahendica,* antiplasmodial, ferruginol, Lamiaceae.

P-33

In Vivo Imaging of Dendritic Cell Migration within Lymphatic Vessels

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Introduction: Dendritic cells (DCs) take up antigen in the periphery and transport it via the lymphatic vessels to the lymph node, where they present it to T cells. As such, DCs play an important role in the initiation of adaptive immunity, for instance during infections or vaccinations. Many DC mediated functions have been well studied to date. However, DC migration from the periphery into the lymph node via the lymphatic vessels has not been investigated in great detail.
Aim: In this project, using confocal microscopy, we intend to image *in vivo,* at cellular resolution, how DCs migrate from the tissue into and within lymphatic vessels.
Methods: A prerequisite to image DC migration within lymphatic vessels *in vivo* are mice, which harbour fluorescent lymphatic vessels and fluorescent DCs in different colours. To achieve this, we bred floxed RFP-mice with mice expressing Cre under the control of the promoter of the pan-endothelial gene VE-cadherin. The offspring (VE-cadherin-Cre x RFP mice) express RFP in both blood vascular and lymphatic endothelial cells. For confocal imaging, we inject *in vitro* LPS-matured YFP-expressing DCs into the ears of anesthetized mice.
Results: We often find adoptively transferred DCs that co-localize with and migrate within lymphatic vessels. Preliminary data suggest, that – unlike the common assumption – DCs are not just driven by flow but also actively crawl within small lymphatic vessels.
Conclusions: The established model will help to answer many relevant questions related to DC migration, namely about the kinetics and directionality of DC migration within lymphatics or the exact location of DC entry into the vessels.

Keywords: Dendritic cells, lymphatic vessels, intravital microscopy, leukocyte migration.

P-34

Absorption of Poorly Water Soluble Drugs from Self-Emulsifying Formulations in the Caco-2 Cell Model Using Biorelevant Media

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Introduction, aims: The permeation kinetics of model poorly water soluble drug danazol formulated as self-emulsifying drug delivery system through Caco-2 cell monolayers was investigated using biorelevant intestinal media. These formulations are commonly used to improve solubility hence bioavailability of this class of drugs. Yet, their effect on intestinal permeability and in particular their potential interaction with simulated intestinal media in terms of permeability is currently poorly understood.
Methods: Danazol was formulated as microemulsion (ME), consisting of tri-, di- and mono-glyceride and surfactant. Caco-2 cells were cultured on Transwell inserts according to standard protocols. Drug permeation across the cell monolayer was studied with the micro-emulsion using as media cell-compatible fasted and fed state simulating intestinal fluids, $FaSSIF_{Caco}$ and $FeSSIF_{Caco}$. Control experiments were performed with the unformulated drug using the biorelevant media as well as with the microemulsion and the unformulated drug in purely aqueous transport medium (TM). Transport was measured in both the apical-to-basal and the basal-to-apical direction and data were evaluated with a kinetic model that provided drug permeability coefficients of the apical and the basal plasma membrane and the partition coefficient of drug between the media and the cell compartment.
Results: Permeability coefficient values of the drug with the ME formulation were smaller than of the unformulated drug. This was true when biorelevant media as well as when purely aqueous transport medium were used. $FaSSIF_{Caco}$ and $FeSSIF_{Caco}$ themselves reduced permeability coefficients compared to TM. On the other hand, both the microemulsion and the biorelevant media increased drug solubility in water. The model-deduced media-to-cell partition coefficients varied in accordance with the solubility of drug in the media. Apical permeability coefficients were generally 1.5 to 4 times greater than basal values.
Conclusions: These results appear to support the view that incorporation of drug in colloidal structures of the ME formulation and of the biorelevant media diminishes its potential to permeate the cell membrane. Whether, in saturated solutions, this decrease in permeability is counterbalanced or even overpowered by the increased solubility of the drug, yielding potentially higher fluxes, is currently under investigation.

Keywords: Danazol microemulsion, caco-2 cells, permeability coefficient.

P-35

SAR Studies on 12-Aza-Epothilones (Azathilones) – A New Structural Class of Microtubule Stabilizers

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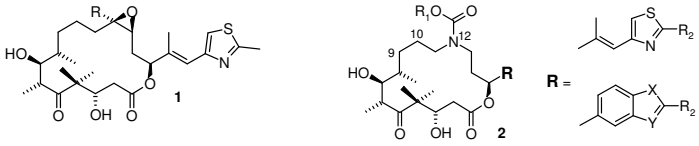
Introduction: Epothilones (Epo's) are bacterial natural products with potent antitumor activity *in vitro* and *in vivo.* At least seven epothilone-derived agents have entered clinical trials in humans. While all of these compounds are closely related structurally to the natural product leads Epo A (1, R = H) and B (1, R = CH_3), our own work involves the design, synthesis, and biological evaluation of analogs with significantly altered structural features, which would represent new lead structures for anticancer drug discovery.
Aims: This paper will discuss the synthesis and biological properties of epothilone analogues of the general structure **2** (R_1 = alkyl; X, Y = N(CH_3), CH=CH, N, R_2 = H, CH_3)



Methods: By means of chemical total synthesis analogs of type **2** were prepared for the evaluation of their biological activity in cell culture assays.
Results: In spite of significant architectural differences between these analogues and natural epothilones, the biological activity of individual compounds from both structural series is within the same potency range as that of Epo A or B. At the same time, some of the typical SAR features of natural epothilones are not reproduced in compounds of type **2.**
Conclusions: We suggest that the tubulin-bound conformation of the herein evaluated azathilones may differ from that of natural epothilones.

Keywords: Epothilone, azathilone, microtubules, SAR.

P-36

A New Synthetic Approach to Side Chain-Modified Analogs of Cyclopropyl-Epothilone B

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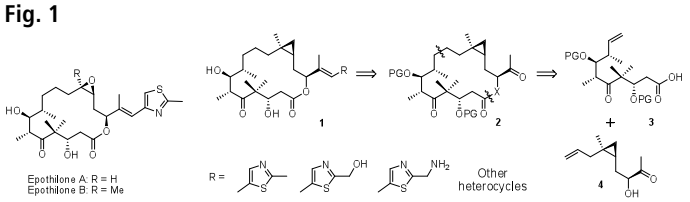
Introduction: Epothilones (Epo's; Scheme 1) are microtubule-stabilizing agents with potent *in vitro* and *in vivo* antitumor activity. Initially isolated from the myxobacterium *Sorangium cellulosum* with Epo A and B as the major variants, they quickly established themselves as important lead structures for anticancer drug discovery [1]. Among numerous other modifications the replacement of the epoxide ring by a metabolically more stable cyclopropane moiety has been shown to be well tolerated or even lead to enhanced cellular potency, and the same is true for a variety of side chain modifications [2].
Aims: In a project that ultimately aims at the construction of antibody-drug conjugates we have now prepared a series of side chain-modified analogues of cyclopropyl-Epo B **1** and evaluated their anti-proliferative and tubulin-polymerizing activity.
Methods: By means of chemical total synthesis analogues of type **1** were prepared to test their ability to inhibit the growth of human cancer cells *in vitro.*
Results:

Fig. 1



The synthesis of analogues **1** is based on a novel and highly flexible approach that relies on late stage introduction of the side chain through Horner-Wittig-Emmons chemistry and ring closure through RCM (Fig. 1). This contribution will discuss the details of the synthesis of macrolactone **2** from building blocks **3** and **4** and its elaboration into the desired target structures.
Conclusions: A new synthetic access to side chain-modified analogs of metabolically stable cyclopropyl-epothilone B analogs **1** has been established.

Keywords: Epothilone, microtubules, SAR, antibody-drug conjugates.

References:
[1] K.-H. Altmann et al. Chem Med Chem 2007; 2: 396.
[2] K.C. Nicolaou et al. J Am Chem Soc 2001; 123: 9313.

P-37

pK_a Determination by ^{1}H-NMR Spectroscopy – An Old Methodology Revisited

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Introduction: pK_a values of acids and bases have an essential impact on organic synthesis, medicinal chemistry and material as well as food sciences. Especially in drug discovery and development, pK_a values are of upmost importance for the prediction of pharmacokinetic and pharmacodynamic properties, for example permeation through biological barriers, interactions with targets or induction of side effects. To date, different methods for the determination of pK_a values are available, including potentiometric, UV-spectroscopic, and capillary electrophoresis techniques. An additional option is provided by nuclear magnetic resonance (NMR) spectroscopy.
Aims: pK_a determinations by ^{1}H-NMR spectroscopy are reported for numerous, but mostly isolated cases. We attempted to fully evaluate the potential of this approach.
Method: The underlying principle is the alteration of chemical shifts of magnetic nuclei in NMR spectroscopy depending on the protonation state of an adjacent acidic or basic site. When these chemical shifts are plotted against the pH, the inflection point of the resulting sigmoidal curve defines the pK_a.
Results: A diverse set of test compounds covering a broad range of pK_a values (1 to 14) was used. Values determined by the NMR approach were compared to reference values obtained with electropotentiometric as well as UV spectroscopic methods and an excellent correlation was observed (R^2 = 0.99). Furthermore, a comparison with computed results showed that the NMR-based approach yielded pK_a values with a lower maximal deviation.
Conclusions: The reliability and utility of the ^{1}H-NMR approach could be demonstrated. It is easily accessible, fast, highly sensitive and does not require large sample amounts. This uncomplicated access to pK_a values can be extremely beneficial for many disciplines, e.g., medicinal chemistry.

Keywords: pK_a determination, ^{1}H-NMR spectroscopy, physicochemical properties, dissociation constant.

P-38

PADMET-Platform: Pharmacokinetic Profiling of Glycomimetics

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Introduction: Carbohydrate-lectin-interactions play an important role in numerous pathological conditions such as inflammation, infection or cancer. However, therapeutic applications using carbohydrates are only successful in rare cases due to their drug-unlikeness. Especially their high polarity limits the oral availability and, once they are in circulation, contributes to a fast renal excretion.
Aims: In order to specifically improve the drug-likeness of carbohydrate lead structures, we evaluated their pharmacokinetic properties with our PADMET-platform. PADMET stands for *p*hysicochemical properties, **a**bsorption, **d**istribution, **m**etabolism, **e**limination, and **t**oxicity.
Methods: The platform is intended to cover the PADMET aspects with assays typically used at early stages of lead development. Assays were built up to determine physicochemical parameters (logD, pK_a, solubility), absorption behavior (with PAMPA and Caco-2 assay), plasma protein binding, and stability towards hepatic metabolism.
Results: A series of potential glycomimetic antagonists of FimH (a lectin located at the tip of bacterial pili of uropathogenic *E. coli*) were characterized with regard to their pharmacokinetic properties. The obtained results were used to choose the most promising compounds for *in vivo* testing, where confirmation of the *in vitro* predictions could be achieved.
Conclusions: The benefits of the PADMET-platform for the development of glycomimetics could be demonstrated by identifying an orally available FimH-antagonist.

Keywords: Physicochemical properties, pharmacokinetic characterization, glycomimetics.

P-39

Coating Pellets of Methionine for Release Ruminal and Intestinal in Sheep

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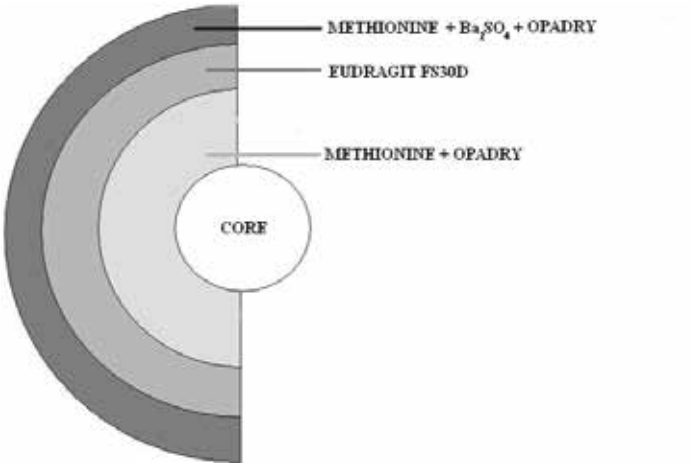
Introduction: Methionine is used in this project because diets with soybean meal or cotton seed are deficient in methionine. Methionine has been considered as the main limiting amino acid for milk protein synthesis in sheep. Ruminally protected amino acids have been used to increase duodenal flow of essential amino acids to improve milk production. On the other hand, nitrogen sources such as amino acids and peptides release in rumen, enhanced growth and efficiency of rumen microbes, microbial proteins that subsequently ruminant take advantage for milk and meat protein synthesis.
Aims: To develop a modified release system for the administration of methionine at the level of rumen and intestine in the ruminant.
Methods: For the development of modified-release system, inert pellets were used as core and several coatings were made at various stages. First, methionine was used with a non-functional polymer (Opadry). Thereafter, the product was covered with a functional polymer (Eudragit FS30D) which dissolves at pH above 7.2 and finally the outermost layer consisted of methionine, an inert polymer (Opadry), talc and barium sulfate to increase the density of the pellet and release in rumen.



Quantification of methionine, as raw material, intermediate products and final product was conducted by UV spectrophotometry according to Moore & Stein (1954). Dissolution tests were conducted during the stages of coating to quantify methionine. The dissolution medium was acetate buffer pH 6, which subsequently changed to 4 and above 7.2.
Results: The amount of methionine in the first stage of the project and targeted to the bacterial flora was 94 mg/g of pellets and the dissolved percentage was 99.3%. The amount of methionine targeted to intestine in the ruminant was 164 mg/g of pellets, which means 99.6% dissolved.
Conclusions: In the dissolution test, almost the total amount of methionine incorporated in the different layers could be quantified, which indicated release in the rumen and intestine. This device would be a new way to provide methionine to rumen and intestine of ruminants. However, this could only be determined when used directly on the animal.

Keywords: Release targeted, pellets, ruminant, methionine, Eudragit FS30D.

P-40

Development, *In Vitro* and *In Vivo* Testing of a Novel Enteric-Coated Sodium Thiosulfate (STS) Tablet

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Introduction: Sodium thiosulfate (STS, $Na_2S_2O_3$) is a well-tolerated drug, which has been used for decades as an antidote for cyanide poisoning, and recently for the treatment of calciphylaxis, a severe calcifying disease. For both these indications, STS is given intravenously. To date, no STS-containing tablet has been developed to overcome the disadvantages of bad taste and gastric acid instability exhibited by oral use of aqueous STS.
Aims: The aims of this study were twofold: (i) development and *in vitro* testing of an enteric-coated STS tablet formulation, and (ii) the *in vivo* verification of improved bioavailability and neutral taste.

Methods: A 300 mg STS-containing tablet was designed, consisting of a STS core, coated with a powder-coating layer, and covered by an outer enteric coating layer. The function of the powder coating layer was to protect the core from heat and acidity applied during the enteric coating process. STS core tablets were compacted with an eccentric tablet press and then put in approximately 160 mg of the powder-coating mixture (MCC 102, FlowLac, HPC-SL) and compacted again. The enteric coating process was performed in a bottom spray fluid bed coater and 6–9% wt Kollicoat MAE 30DP was applied for the enteric coating layer. Acid resistance and STS release was tested *in vitro* with a dissolution tester (USP Apparatus II). In a first *in vivo* test between 1 and 8 tablets were taken by 3 healthy volunteers, and thiosulfate concentrations were determined in blood and urine by fluorescence-based HPLC.
Results: Tablet properties and STS release were in accordance with Ph. Eur. 5.0 and USP 31. *In vitro,* less than 10% STS was released within 2 h in pH 1.2, whereas 100% of STS were released thereafter within 45 min in pH 6.8 with a very low intra- and inter-batch variability. *In vivo,* the tables were of neutral taste and without detectable side effects. Thiosulfate peak urine levels were usually observed later than 10 h post ingestion.
Conclusions: A novel enteric coated STS tablet was developed. *In vitro* as well as first *in vivo* tests indicate the potential suitability of this tablet for clinical use. Clinical studies are now needed to closer define the pharmacokinetic properties of this tablet.

Keywords: Sodium thiosulfate, STS, enteric coating, calciphylaxis.

P-41

Delivery of Intact Functional Proteins Using Non-Invasive Transdermal Iontophoresis: An Alternative to Parenteral Administration

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Introduction, aims: To demonstrate the feasibility of using transdermal iontophoresis to deliver intact functional proteins non-invasively across porcine and human skin. Experiments were conducted using two model proteins, Cytochrome c (Cyt c;12.4kDa) and Ribonuclease A (RNAse A;13.6kDa), and a larger protein with potential therapeutic applications – basic Fibroblast Growth Factor (bFGF;17.4kDa).
Methods: Experiments were performed using vertical diffusion cells; the donor chamber contained 1mL of protein solution (0.7mM Cyt c (pH 8.2), 0.7mM RNAse (pH 6), and 0.029mM bFGF (pH 7.4)). Co-formulation with 15mM acetaminophen, a neutral molecule, enabled determination of the relative contributions of electromigration and electroosmosis to transport. Constant current iontophoresis (0.5 mA/cm^2) was performed for 8 h and the receiver compartment sampled hourly. After current application, bound protein was extracted from the skin. Electrotransport of Cyt c, RNAse A and bFGF was quantified by reversed phase HPLC with UV detection, a methylene blue-based enzymatic assay and ELISA, respectively. In addition to quantifying mass transport, the assay for RNAse A could simultaneously demonstrate that enzymatic activity was retained post-iontophoresis.
Results: The three proteins were chosen based on their globularity (global curvature) and pI. Cumulative iontophoretic permeation of Cyt c was 923.0 ± 496.1 µg/cm^2. No Cyt c degradation peaks were observed in the chromatograms confirming that it remained intact post-iontophoresis. Cumulative permeation of RNAse A was 170.7 ± 92.1 µg/cm^2. The structural integrity was confirmed by SDS-PAGE and MALDI-TOF spectra (Fig. 1). The methylene blue assay confirmed that RNAse A retained activity after skin transit. ELISA showed that bFGF was delivered across porcine skin; cumulative permeation and skin deposition were 16.0 ± 6.6 and 77.7 ± 37.4 µg/cm^2, respectively. Furthermore, bFGF delivery across porcine and human tissue was found to be statistically equivalent (16.0 ± 6.6 and 12.6 ± 1.3 µg/cm^2, respectively). Co-iontophoresis of acetaminophen showed that electromigration was the dominant electrotransport mechanism, accounting for ≥90% of total Cyt c and RNAse delivery and ~75% of bFGF transport (Fig. 2).

Fig. 1

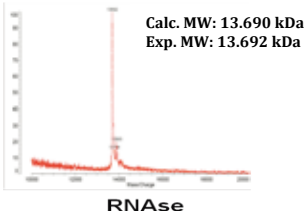
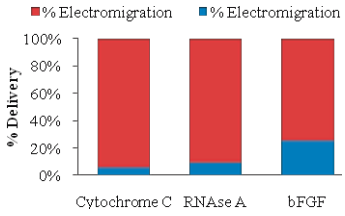


Fig. 2



Conclusions: The results demonstrate that iontophoresis can deliver intact, functional proteins non-invasively across the skin. This may be of relevance for local and systemic therapy; the technology may provide a realistic alternative to parenteral administration.

Keywords: Functional proteins, transdermal iontophoresis, non-invasive skin delivery.

P-42

Constant Current Iontophoresis to Deliver Active RNAse (13.7 KDA) Across Intact Porcine Skin

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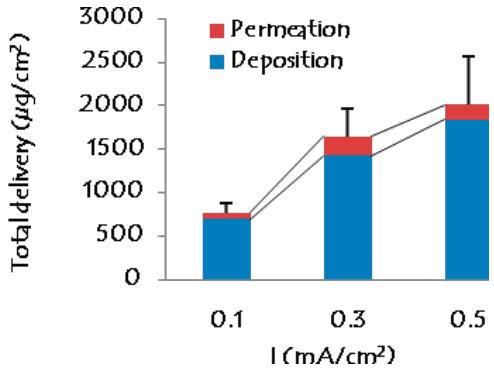
Introduction, aim: To demonstrate the feasibility of using constant current iontophoresis to deliver active RNAse (13.7 kDa) across intact porcine skin.
Methods: Dermatomed skin (thickness 750 µm) was clamped in three-compartment vertical flow-through diffusion cells. The donor compartment, containing 1 mL of RNAse solution (0.7 mM; in 27.7 mM HEPES buffer (pH 6) containing 15 mM acetaminophen), was connected to the anode by a salt bridge. The anode, cathode and receiver compartments were filled with 25 mM HEPES/133 mM NaCl (pH 7.4). Constant current iontophoresis was performed for 8 h and samples collected hourly. Upon terminating current application, RNAse was extracted from the skin samples in 10 mL HEPES-NaCl buffer (pH 7.4) for 18 h. It was quantified by means of an activity assay using methylene blue (MB)-RNA complex. Briefly, 100 µL of RNA solution (10 mg/mL in 100 mM HEPES/2 mM EDTA pH 7.5) and 1 mL of MB solution (0.01 mg/mL in 100 mM HEPES/2 mM EDTA pH 7.5) were incubated for 10 min in the dark to produce an MB-RNA complex; then, 500 µL of sample (or standard) was added and the decrease in absorbance at 688 nm was measured.
Results: It was shown that current application enabled RNAse transport across intact skin; co-iontophoresis of acetaminophen confirmed that electromigration was the predominant transport mechanism. A 3-fold increase in current density from 0.1 to 0.3 mA/cm^2 resulted in a proportional increase in RNAse permeation (70.37 ± 59.77 and 224.37 ± 72.34 µg/cm^2, respectively); there was a 2-fold increase in total delivery (that is, permeation + skin deposition) from 764.97 ± 116.75 to 1643.85 ± 320.36 µg/cm^2, respectively (Fig. 1). Despite significant RNAse accumulation in the membrane, there was little electroosmotic inhibition (IF = 1–2). There was no increase in delivery upon further increasing current density from

0.3 to 0.5 mA/cm^2. Decreasing RNAse concentration in the formulation from 0.7 to 0.35 mM did not impact on total RNAse delivery (1829.61 ± 345.94 and 2567.13 ± 357.57 µg/cm^2, respectively).

Fig. 1



Conclusions: The results demonstrate that iontophoresis can be used for the non-invasive delivery of a functional protein across intact skin.

Keywords: Active RNAse, constant current iontophoresis, non-invasive porcine skin delivery.

P-43

HPLC Profiling of Coptidis Rhizoma Extract for hERG Channel Inhibitors of Natural Origin

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Introduction: The hERG channel conducts the rapid delayed rectifier K^+ current (I_{Kr}). In the myocardium, this current accelerates the repolarization of the action potential. HERG channel inhibition delays repolarization and prolongs the cardiac action potential and QT interval which can lead to sudden death due to *torsades de pointes* ventricular tachycardia and ventricular fibrillation. Several drugs have been withdrawn from the market due to hERG channel inhibition. HERG is therefore an important antitarget in drug development. Comparatively little is known about hERG channel blockers of natural origin.
Aims: For the search for hERG channel inhibitors of natural origin, we screened a library of herbal extracts derived from medicinal plants which are listed in either the European or the Chinese Pharmacopoeia.
Methods: We established an HPLC-based profiling approach by combining HPLC-microfractionation with online and offline spectroscopy, and an automated two-microelectrode voltage-clamp assay on *Xenopus laevis* oocytes expressing heterologous hERG channel.
Results: Among the extracts tested, the methanolic extract of the TCM herbal drug Coptidis rhizoma (*Coptis chinensis* Franch., Ranunculaceae) reduced the peak tail hERG current by 32% at 100 µg/mL. HPLC-based activity profiling pointed towards berberine as the active constituent. However, activity could not be confirmed with a reference sample of berberine tested at 100 µM. Subsequent HPLC-DAD-MS analysis showed that berberine collected by microfractionation of *Coptis* extract had been, in part, transformed to active dihydroberberine. Formic acid added to the HPLC mobile phase to reduce peak tailing of protoberberine alkaloids acted as a reducing reagent according to the mechanism of the Leuckart-Wallach reaction. Structurally related quaternary protoberberines, and di- and tetrahydroberberine were tested for their potential as hERG channel blockers. Quaternary protoberberines were marginally active, and substitution pattern at positions C-2, C-3, C-9 and C-10 had moderate influence on hERG channel activity. Dihydroberberine showed the highest activity.
Conclusions: The hERG channel inhibitory activity of the active time-based microfractions could be mainly assigned to the processing artefact dihydroberberine rather than to the previously reported hERG channel blocker berberine.

Keywords: hERG channel inhibition, herbal extracts, *Coptis chinensis,* HPLC-based activity profiling, protoberberine alkaloids.

P-44

Non-Covalent PEGylation: Novel Excipients Stabilizing Salmon Calcitonin Against Aggregations

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Introduction: During all stages of protein drug development, aggregation is one of the most often encountered problems compromising the drug's safety and efficacy. Protein aggregation, being a very complex process, may depend on a multitude of environmental factors (e.g., temperature, pH, ionic strength or protein concentration), applied stresses (e.g., shaking, freezing or thawing), and the structure of the protein itself. For many proteins, a nucleation-dependent mechanism has been described, during which aggregation-prone folding/unfolding intermediates are formed by conformational changes. Those favour protein/protein interactions, and thus initiate aggregation, by liberation and approximation of hydrophobic patches, which are usually buried inside the intact protein. PEGylation, the covalent attachment of poly(ethylene glycol) to therapeutics, is known, besides others, to reduce aggregation due to shielding of hydrophobic patches on protein surfaces. A major drawback of PEGylation is the conjugation step for which most often chemical strategies are employed.
Aims: The purpose of this project was the development of PEG polymers, which are able to act as stabilizing excipients by non-covalently linking the PEG to a protein via hydrophobic interactions. Here we present the new method of stabilizing salmon calcitonin, as a model biopolymer, against aggregation combining the positive effects of PEGylation (reduced aggregation), while circumventing chemical processing of the drug.
Methods: Several polymers consisting of a hydrophobic headgroup attached to PEG-polymers of different molecular weight were synthesized and characterized by NMR, FTIR, MALDI-TOF and various other spectroscopic techniques. The influence of these excipients on the aggregation of salmon calcitonin in various buffer systems was tested by following changes in Nile Red fluorescence and turbidity over time.
Results: Depending on the type of headgroup and molecular weight of the PEG-polymer, the onset of aggregation was prolonged and the final degree of aggregation reduced.
Conclusions: The aggregation of salmon calcitonin is changed in presence of our polymers, leading to a prolongation in the onset of aggregation and a less aggregated state of the biopharmaceutical.

Keywords: Salmon calcitonin, non-covalent PEGylation, stabilizing excipients.

P-45

Fate of TLR-2 Agonist Functionalized pDNA Nanoparticles Upon Deposition at the Bronchial Epithelium *In Vitro*

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Introduction: The present strategy for the formulation of new plasmid DNA (pDNA) vaccines is to include highly purified synthetic adjuvants increasing the vaccine´s immunogenicity. Moreover, for effective vaccination, the adjuvant needs to be co-delivered with the pDNA vaccine within the same particulate system. In order to address that necessity, we recently synthesized a novel chitosan-based co-polymer *CM-TMC-g-PEG-Pam$_3$Cys* containing the *Pam$_3$-Cys* moiety, which activates the human innate immune system via Toll-like receptor-2 (TLR-2). Subsequently, *Pam$_3$Cys* decorated nanoparticles (NP) were prepared by complex coacervation between *CM-TMC-g-PEG-Pam$_3$Cys* and a model plasmid DNA (pGFP) expressing the green fluorescence protein (GFP). Interestingly, we observed that *Pam$_3$Cys* (TLR-2 agonist) decoration of pGFP NP correlated with a 10-fold higher immunogenicity, when interleukin 8 (IL-8) secretions were quantified from differentiated THP-1 human macrophages.
Aims: In order to study further the potential of the new particulate system for pulmonary pDNA vaccination, pDNA loaded NP were aerosolized via a microsprayer onto the surface of a triple cell culture system of the human bronchial epithelium. Uptake of NP by epithelial and immune cells (blood monocyte-derived dendritic cells (MDDC) and macrophages (MDM)) was visualized by confocal laser scanning microscopy (CLSM). Moreover, secretions of immune cytokines (IL-8 and tumor necrosis factor-α (TNF-α)) from the triple co-culture cell system were quantified.
Methods: To a new chitosan derivative, 6-*O*-carboxymethyl-*N,N,N*-trimethylchitosan *(CM-TMC),* the *Pam$_3$Cys* moiety was grafted through a polyethylene glycol (PEG) spacer. In a second step, *Pam$_3$-Cys* decorated pGFP NP were formulated and then aerosolized by using a microsprayer onto the triple cell culture model. After 24-h incubation, CLSM of apical as well as basolateral compartments were performed followed by ELISA of IL-8 and TNF-α secretions in basolateral media.
Results: CLSM demonstrated that transfer of chitosan-based pGFP NP into basolateral MDDC as most competent immune cells took place. Although no significant difference in uptake pattern was observed for TLR-2 agonist modified (*CM-TMC-g-PEG-Pam$_3$Cys* NP) and unmodified (*CM-TMC* NP) systems studied, ELISA of IL-8 and TNF-α exhibited clearly that TLR-2 agonist decoration enables a significant higher immunogenicity.
Conclusions: This study further confirms the benefit of covalent adjuvant decoration of chitosan-based pDNA NP by using a TLR-2 agonistic moiety. Further studies ongoing in our lab will embrace the use of presented co-polymer for delivery of relevant DNA vaccines in terms of pulmonary vaccination.

Keywords: Nanoparticle, pulmonary DNA vaccination, chitosan derivative, triple cell culture model.

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"Party Drugs" Testing on the Dance Floor: Equipment, Methods and Results

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Introduction: Since 1998 the mobile lab unit of the Office of the Cantonal Pharmacist is testing "party drugs" on the dance floor. During this prevention project in cooperation with "Streetwork Zurich" and "Contact Berne" the lab team has tested over 1600 samples at more than 100 occasions – from small club venues to big events like the "Streetparade" in Zurich.
Aims: The intention of this contribution is to present the interdisciplinary cooperation of the lab staff with social workers, the equipment and methods used by the lab and an updated survey of the testing results.
Methods: The configuration of the newly established 3rd generation of the mobile high-tech lab (proprietary development based on commercially available components) includes 2 WLAN-controlled HPLC-DAD instruments, precision balance and ultrasonic extractor mounted on four custom made steel framed racks on wheels. Their small footprint allows for easy transportation as well as rapid and flexible installation, necessary for attending not only big events but also small clubs. The lab is operated by two experienced technicians who can handle up to 6 samples per hour. Sample processing includes digital documentation as well as simultaneous qualitative and quantitative chemical analysis by HPLC-DAD. Routinely more than 50 active substances can be reliably characterized. During the laboratory analysis, which is free of cost, a social worker accomplishes a counseling session including a structured interview based on an anonymous questionnaire. If the lab detects unknown or dangerous compounds, hazardous combinations or high doses, the potential consumers and – if necessary – a greater public is warned with appropriate means (see www.saferparty.ch and www.raveit-safe.ch).
Results: For many years MDMA (Ecstasy) has been and still is the preferred party drug. Since 2005, the increased emergence of fake ecstasy pills with meta-chlorophenylpiperazine (m-CPP) as the active ingredient with very unpleasant side effects has worried the consumers. Today cocaine is the third most frequently analyzed compound after MDMA and amphetamine. Since 2003, a wave-like growing number of cocaine samples in combination with a price decline have been observed. Simultaneously a decrease of the cocaine content from more than 50% to less than 30% mass fraction was detected over the years. Remarkable is the fact that harmful adulterants are now found more frequently, such as the illicit pain killer phenacetine or the veterinary anthelmintic levamisol. A new trend was observed about two years ago. New compounds, so-called "designer drugs", increasingly appeared at parties. Examples are mephedrone, methylone and butylone which could be identified recently. These drugs are derivatives of known psychoactive compounds but they are not (yet) listed as scheduled drugs in the narcotic law.
Conclusions: On an individual and immediate base the onsite drug testing facilitates the contact with the target user group including counseling. It also allows for scientific sound advice concerning consumption risks as a base for prevention and harm reduction. On a long-term view, attending about 10–12 parties per year gives an insight into the situation on the illegal market regarding new drugs and changes in consumption trends. Thereby possible health risks are identified as the mentioned examples illustrate.

Keywords: Designer drugs, drug prevention, ecstasy, mobile laboratory, HPLC.

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Transdermal Iontophoretic Delivery of Niosome-Encapsulated Insulin

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Introduction: The combination of iontophoresis and vesicle entrapment of proteins may provide a non-invasive method for insulin delivery. In this study we evaluated the iontophoresis and bilayer-charge effects on the transport of insulin through the rat abdominal skin.
Aims: Evaluation of the effect of two transport enhancing methods, vesicular entrapment and ionotophoresis, on the non-invasive delivery of insulin through the skin was the main goal of this study.
Methods: Different neutral, anionic (containing dicetylphosphate) and cationic (containing stearylamine) niosomes were prepared by two methods, dehydration rehydration (DRVs) and film hydration. The vesicles' volume diameter and protein encapsulation efficiencies were measured. The protein integrity of insulin was analyzed by sodium dodecyl sulfate polyacrylamide gel (SDS-PAGE) electrophoresis. Shaved abdominal rat skin was used for iontophoresis of insulin in free or niosome-entrapped formulations. Platin electrodes were used for induction the potential difference between the donor and receptor compartments in Franz diffusion cell.
Results: The encapsulation efficiency of insulin was 31–35% in different formulations. Gel electrophoresis of prepared formulations showed insulin structure does not change during preparation procedures. The neutral vesicles have shown more entrapment efficiencies with no significant difference ($p > 0.05$). Vesicular systems depend on their volume diameters, composition and charge had different effects on insulin transport. During the passive insulin transport, the amount of the protein receptor compartment was about 0%. The iontophoresis increased the transport of insulin as high as 12% in negatively-charged vesicles, 29% in positive ones, 52% in neutral ones and 5% in insulin solution during 6 h.
Conclusions: Iontophoresis and niosome encapsulation resulted in a significant increase in transport of insulin through rat skin in comparison with passive insulin diffusion, hopefully a new drug delivery system for insulin.

Keywords: Insulin, iontophoresis, niosome, rat skin.

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Solid Oral Nanoparticulate Formulation

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Introduction: To overcome the solubility problem for class II compounds, special delivery systems such as liposomes, microemulsions, solid dispersions, and inclusion complexes using cyclodextrins show reasonable success. Nonetheless, these formulations have limitations, which include limited drug loading capacity, use of organic solvents for preparation, important residual solvent content, thermal sensitivity, and difficult control of particle size and size distribution during processing. These characteristics can affect drug particle stability during storage, powder flow properties, and the therapeutic efficiency of the delivery system. Another promising formulation approach is by comminution of the drug particles to the sub-micron range. The main advantage of nanocrystals for oral delivery is their increased dissolution rate and, hence, enhanced oral absorption, which is related to the increased specific surface area of the particles. However, because of marketing preference (patient convenience) as well as physical (e.g. Ostwald ripening and agglomeration) and chemical (e.g. hydrolysis) instability problems associated with nanosuspensions, their drying should be considered as essential step for producing the final oral dosage form.
Aims: The aim of this work was to develop a solid oral dosage form (capsule, tablet) from a nanosuspension of a poorly water-soluble BCS class II type drug compound (Drug X).
Methods: Drug X was first comminuted by wet media milling to obtain a nanosuspension. The nanosuspension was then transformed into a dry product through spray-granulation drying, where the nanosuspension was layered onto a water soluble particulate carrier. The particle size of the nanoparticles after re-dispersion of the dried product was measured using photon correlation spectroscopy (PCS). SEM and EDX were used for the characterization of dried nanoparticles.
Results: Particle sizes of spray-layered nanoparticles upon re-dispersion were larger compared to those in the original nanosuspensions, but still below 400 nm. SEM/EDX mapping showed that the nanoparticles were layered like a coat around the large carrier particles – the shape of the nanocrystals being still visible in the SEM images. Dissolution testing of capsules containing the spray-layered granules with a particular surfactant depicted superior release compared to the micronized formulation and to the other nanoformulation with an alternative surfactant.
Conclusions: Spray-layering represents a feasible drying technology for nanosuspensions, which results in granules with high drug loads up to >40%, acceptable re-dispersion properties, and overall good further downstream processing properties.

Keywords: Spray-layering, nanosuspensions, carrier excipients, re-dispersibility, SEM/EDX mapping.

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Capillary Electrophoresis/Frontal Analysis (CE/FA) Assays for the Determination of Binding Constants

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Introduction: Drug-plasma protein interactions have a significant impact on both pharmacokinetics (*i.e.,* absorption, distribution, metabolism, and elimination) and pharmacodynamics (pharmacological effects). It is therefore of high interest to evaluate this parameter during the drug development process. Capillary electrophoresis (CE) profiling itself is an interesting tool of drug-protein binding characterization due to its low reagent consumption and possibility to work under near-physiological conditions. The most interesting mode of CE to study biomolecular interactions is frontal analysis (CE/FA). However, as no rigorous guidelines are given in the literature, some confusion in conducting CE/FA experiments has emerged.
Aims: The present study describes the most important steps to take into consideration when building up new CE/FA binding assays, *i.e.,* the choice of the buffer and applied voltage, the evaluation of protein adsorption onto the capillary walls, the choice of the injection volume, the verification of co-migration of the protein and drug-protein complex, and finally the choice of the drug and protein concentrations.
Methods: CE in frontal analysis mode is used to asses drug-plasma protein binding.
Results: The developed method can serve as a checklist of the key parameters that need to be addressed for successful and reliable

interaction studies. The strengths of the binding constants for nine selected drugs (basic, neutral, acidic substances) to albumin (the most important plasma protein) were also measured and ranged from log K_a 2.9 to 5.4 which include most of the drug- albumin interaction systems.
Conclusions: The CE/FA method developed in this work is an easy and simple way of assessing binding constants (K_a) and interaction stoichiometries, with a relatively high-throughput and low reagent consumption.

Keywords : Capillary electrophoresis, frontal analysis, binding constants, plasma proteins.

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How a Detailed Description of Hydrophobicity Can Enhance the Quality of Docking: Application to the GOLD Strategy

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Introduction: GOLD is a reliable docking software well known in the field of drug design. The posing algorithm was originally developed to dock small polar ligands in hydrophilic pockets, as such the search engine focuses mainly on hydrogen bonding pattern recognition. At early stages of docking, it creates a list of so-called "hydrophobic fitting points" inside the protein cavities that steer the positioning of ligand hydrophobic moieties. The generation of fitting points is based on Lennard Jones potentials between a carbon probe and each atom of the residues delimitating the binding sites. Since the intermolecular forces encoding hydrophobicity are not only of steric nature, it is not surprising that this program may fail in situations in which the hydrophobicity acts as major driving force.
Aims: The present study aims at better describing apolar regions in protein pockets through a new tool, the *MLP-filter,* that properly guide ligand moieties towards hydrophobic areas during the GOLD docking runs.
Methods: The *MLP-filter* retains only genuine hydrophobic fitting points, in the binding pockets, matching the rigorous definition of lipophilicity given by the Molecular Lipophilicity Potential (MLP), which relies on experimentally determined n-octanol/water partition coefficients (log $P_{o/w}$).
Results: The new method was tested on a high-quality test set of 84 protein-ligand complexes selected from the PDBbind database with an emphasis on diversity and filtered in order to exclude the polar binding pockets for which GOLD is known to be suited. The application of the MLP-filter resulted in a significant increase in docking accuracy compared to the standard docking procedure (~20% of docking improvement).
Conclusions: The *MLP-filter* is a new tool that can be easily added to GOLD docking procedure for a more rigorous description of hydrophobicity. The promising results lay the foundations for future applications of the *MLP-filter* in various virtual screening projects and fragment-based protocols.

Keywords: Docking, molecular lipophilicity potential, GOLD.

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Studying the Mechanisms of Cannabinoid Receptor Trafficking and Subcellular Receptor Distribution in Peripheral Cells

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Introduction: Emerging evidence implicates a role of the endocannabinoid system in a wide variety of physiological and pathophysiological processes, including metabolic regulation, pain, anxiety, immunomodulatory functions and bone growth. While cannabinoid receptor type 1 (CB_1) is densely distributed on neuronal cells and is responsible for the psychotropic effects of cannabinoids, cannabinoid receptor type 2 (CB_2) is mainly expressed by immune cells like monocytes/macrophages, hematopoietic cells and osteoclasts, being closely linked to the regulation of chronic and acute inflammatory processes, at least partially due to modulation of cytokine expression. Since cannabinoid receptor agonists/antagonists provide a tool to interfere in pathological processes, the pharmacological interest in substances influencing cannabinoid receptor signaling is high. Our lab discovered a "molecular switch" allowing to trigger the trafficking of CB receptors from the plasma membrane to intracellular compartments and *vice versa,* depending on the cell type, thereby implicating a new potential regulatory mechanism for CB receptor signaling, as receptor surface density is a key regulatory mechanism for G protein-coupled receptor (GPCR) activity, influencing strength and duration of signal transduction into the cell.
Methods: Lipid raft preparation, co-immunoprecipitation, immunofluorescent microscopy, subcellular fractionation, FACS.
Results: CB receptor trafficking in peripheral cells is cell type-dependent and can be elicited by endogenous lipids and protein phosphatase modulators. Membrane fractionation studies showed that in the promyelotic HL60 cell line both CB_1 and CB_2 receptors could be detected in the lipid raft fraction of the plasma membrane. In the same cell line CB_1 and CB_2 could be co-immunoprecipitated from the whole plasma membrane fraction and from the lipid raft fraction. Transfection of the fibroblastic cell line NIH3T3 with a CB_2-GFP construct revealed a perinuclear localization of the receptor and immunofluorescent staining of breast cancer cells showed a cytoplasmatic and nuclear distribution of the CB_2 receptor.
Conclusions: Lipid raft localization and co-immunoprecipitation suggest a possible regulation of cannabinoid receptor signaling within these microdomains in peripheral cells. The view of cannabinoid receptors solely located at the plasma membrane before desensitazation and sequestration (in the case of neuronal CB_1) upon agonist binding has to be widened in the context of subcellular distribution of the receptor depending on the cell type

Keywords: Cannabinoid receptor, G-protein coupled receptor (GPCR), lipid raft, co-immunoprecipitation.

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Effects of MDMA (Ecstasy) on Circulating CT-Pro Vasopressin (Copeptin) in Healthy Humans

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Introduction: 3,4-Methylenedioxymethamphetamine (MDMA, 'Ecstasy') use has been associated with hyponatremia which could be due to inappropriate secretion of plasma arginine vasopressin (AVP)

particularly in women [1]. We investigated effects of MDMA on AVP and CT-pro AVP (copeptin) [2] secretion and on plasma and urine osmolality and sodium levels in a laboratory setting in healthy men and women.

Methods: We used a randomized placebo-controlled cross-over design with placebo and MDMA (125 mg) in balanced order in 16 healthy subjects (8 males and 8 women). We assessed plasma AVP, sodium, and osmolality plus urine sodium and osmolality before and 120 min after drug administration. Copeptin levels were assessed before, 60 and 120 min after drug administration. Subjects were not engaged in any physical activity and drinking was ad libitum. AVP and copeptin were assessed by immonoassays [2]. All female subjects were investigated during the follicular phase (day 2–14) of their menstrual cycle when the reactivity to amphetamines and osmotic sensitivity are similar to men. ANOVAs with drug (placebo vs. MDMA) and time (baseline, 60 and 120 min) stratified for gender and followed by Tukey posthoc tests were used to assess differences in drug effects.

Results: MDMA significantly elevated plasma copeptin levels at 60 min ($p<0.001$) compared to placebo in women but not in men [drug $\times$ time $\times$ gender interaction: $F(2,28) = 3.74$; $p<0.05$]. A similar non-significant trend was observed for drug effects on AVP levels. MDMA increased urine osmolality [drug $\times$ time interaction: $F(1,12) = 5.73$; $p<0.05$; drug $\times$ time $\times$ gender interaction: NS] indicating elevated renal water retention. MDMA did not alter plasma sodium levels or plasma osmolality.

Conclusions: MDMA increased circulating copeptin, a marker for AVP system activation, in women but not in men. This sex-difference in MDMA-induced AVP secretion may explain why hyponatremia is typically reported in female Ecstasy users [1].

Keywords: MDMA, 3,4-methylenedioxymethamphetamine, Ecstasy, AVP, CT-pro AVP, copeptin, hyponatraemia.

References:

[1] J. Rosenson, C. Smollin, K.A. Sporer, P. Blanc, K.R. Olson. Ann Emerg Med 2007; 49:164-71.

[2] N.G. Morgenthaler, J. Struck, C. Alonso, A. Bergmann. Clin Chem 2006; 52:112-9.

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