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at the
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On behalf of CRS Germany, I cordially welcome all our members and guests at our 2013 annual meeting in Ludwigshafen.

This meeting links a long fruitful and successful past of CRS Germany with the present topic of control-led drug delivery. In a time with numerous possibilities to engage in options for career advancement – be it through congresses, workshops, meetings, the internet or other sources - the CRS annual meeting is filling an important niche: It is specifically designed for the advancement and active participation of our PhD students, who are on their way to becoming independent scientist, collectively building our future in controlled drug delivery. We know this is an extremely important but not an easy step for students as arguably even the friendly atmosphere of our congress is very new and a stretch for many whose prior reach has been limited to one’s own department so far. However, through an oral presentation, a poster or by active participation in scientific discussions – CRS Germany aims at providing a platform within which this group of young scientists can excel. The numerous feedbacks we received from previous events confirm this impact. We are gratified for all the work and effort so many have invested into the organization of our meetings. Without this dedication and vision, CRS Germany could not exist.

This year, we are particularly indebted to AbbVie and more specifically our colleague and acting vice president Dr. Martin Bultmann. AbbVie is sponsoring and hosting our 2013 event and has organized an extremely interesting program, enriched by a suite of workshops, while keeping the focus on providing a platform to PhD students to take their first steps with the broader community. On behalf of the German Chapter, I would like to cordially thank the organizers of the 2013 congress.

Welcome to CRS Germany, welcome to our 2013 congress.

Prof. Dr. Dr. Lorenz Meinel

President
Program
Abstracts
Introduction
The efficacy of antitumor chemotherapy is limited due to the dose dependant side effects, caused by non specific targeting of the tumor tissue. In attempt to transform this highly acute therapeutic problem into a tightly efficient drug delivery we propose to combine two efficient approaches: encapsulation of doxorubicin in liposomes and targeting with high affine vector molecules [1, 2]. We have selected a number of antibodies very promising for selective tumor therapy and conjugated them to the liposomal surface [3].

Materials and Methods
The immunoliposomes were prepared by phase inversion and membrane extrusion, drug loading and conjugation of antibodies was performed simultaneously followed by gel filtration purification step. Size determination was done with dynamic light scattering. The specific targeting was evaluated with flow cytometry. The antitumor efficacy of doxorubicin loaded immunoconstructions was tested in antigen expressing cell lines.

Results and Discussion
The sterically stabilized ICO-1 and ICO-25 immunoliposomal constructions of 152±10 and 140±5 nm size with 94 % drug loading were successfully prepared and tested in vitro. The specific HLA-DR and MUC-1 antigens reached 96±3 and 97±2% targeting selectivity respectively. The targeted liposomal constructions expressed pronounced cytotoxic activity towards antigen-positive target cells of T-47D and SKOV3, being significantly more effective than the unmodified doxorubicin loaded liposomal formulation (p < 0.05).

References
Sildenafil base and citrate-loaded SLN: 
*In vitro* and *ex vivo* evaluation

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**Introduction**

The aim of this study was to investigate the potential cytotoxicity of solid lipid nanoparticles (SLN) loaded with sildenafil. The SLN were tested as a new drug delivery system (DDS) for the inhalable treatment of pulmonary hypertension in human lungs.

**Methods**

Plain SLN as well as SLN loaded with sildenafil citrate or base (0.1% and 1% of a 30:70 phospholipid:triglyceride lipid matrix, respectively) were manufactured using a customized microchannel approach [1] with a double orifice. All SLN were investigated *in vitro* in human alveolar epithelial cell line (A549) and mouse heart endothelium cell line (MHEC5-T) as well as *ex vivo* in rat precision cut lung slices (PCLS) and precision cut heart slices (PCHS). SLN were applied in a concentration range of 0-5000 µg/ml of lipid matrix. The cell viability was evaluated in *in vitro* and *ex vivo* models by MTT assay.

**Results**

Sildenafil-loaded SLN with particle size of approx. 180 nm and monomodal particle size distribution were successfully manufactured and were stable up to 3 months. LD₅₀ values for A549 cells and PCLS were found to be in the range of 1100 to 1700 µg/ml, while for MHEC5-T cells and PCHS values were found between 1500 to 2700 µg/ml. PCHS showed slightly higher LD₅₀ values in comparison to PCLS. Heart slices could be a useful novel *ex vivo* model for testing toxicity in heart tissue. Considering the toxicological aspects, sildenafil-loaded SLN could have potential as drug delivery system to lungs in the treatment of pulmonary hypertension via inhalation route.

**References**

Differentiation of silicone oil droplets and protein particles by MFI and RMM

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Introduction
Our study aimed to comparatively evaluate Micro-Flow Imaging (MFI) and the recently introduced technique of resonant mass measurement (Archimedes, RMM) as orthogonal methods for the quantitative differentiation of silicone oil droplets and protein particles in the submicron and micron size range. This distinction is highly relevant for the development of biopharmaceuticals, in particular for products in prefilled syringes.

Materials and Methods
The marketed products Humira® and Enbrel® (liquid formulations, prefilled syringes) were analyzed by MFI and RMM to gain insight into relevant droplet/particle levels. Based on those results, samples of artificially generated silicone oil droplets and protein particles were prepared and analyzed both separately as well as in defined mixtures. The concentration of silicone oil droplets and protein particles in the mixtures was determined by (i) the built-in MFI software solution “find similar”, (ii) a customized MFI filter developed specifically for this study, and (iii) the technique of RMM. These determined concentrations were compared to the theoretical concentration for different types of IgG particles and for various droplet/particle ratios.

Results and Discussion
Artificially generated silicone oil droplets and protein particles provided concentrations of \(2 \times 10^5\) to \(6 \times 10^5\) counts/mL (> 1 µm), similar to the analyzed marketed products. The built-in MFI software solution proved to be suitable to discriminate between silicone oil droplets and protein particles for sizes above 2 µm at moderate droplet/particle ratios (70:30 – 30:70). The customized filter was more reproducible than the “find similar” function and enabled reliable discrimination also for more extreme mixing ratios (90:10 – 10:90). RMM showed highly accurate discrimination in the size range of about 0.5 to 2 µm independent of the ratio between silicone oil droplets and protein particles, provided that a sufficient number of particles (> 50 counted particles per species, i.e. in this case about 300,000 particles/mL) was counted.

Conclusion
We recommend combining MFI and RMM, two techniques with a fundamentally different measurement principle, for a comprehensive analysis of biopharmaceuticals potentially containing silicone oil droplets and protein particles in the submicron and micron size range.
Alternative preparation of parenteral emulsions – in vitro physiological tolerability

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Introduction
Dual asymmetric centrifugation (DAC) was recently investigated as an alternative preparation method for parenteral fat emulsions [1]. Advantages of DAC are the economy of time, working in a closed system, possibility of producing small sample sizes and therefore improving screening processes. As for the DAC shear forces appear not as high as in other techniques, e.g. high pressure homogenization, processing of sensitive drugs is possible [2]. However, for production of fat emulsions, due to the lower shear forces in DAC, an increased amount of emulsifier-mixture was needed to produce samples with droplets in the submicron range. The physiological tolerability of these emulsions was investigated.

Materials and Methods
The emulsifier-mixtures were composed of soy lecithin (phosphatidylcholine content \( \geq 75 \% \)), sodium oleate and/or poloxamer 188. Samples contained 5 % emulsifier-mixture, 10 % soybean oil and an aqueous phase isotonized either with glycerol or sorbitol.

Determination of the droplet size distribution was carried out by using a Mastersizer 2000 with submicron instrumentation (Malvern).

Osmolality of the samples was measured with a Semi-Micro Osmometer (Knauer).

The hemolysis assay was modified after Bock and Müller [3].

Results and Discussion
The emulsions differed in type and composition of emulsifier-mixture, but not in emulsifier content.

Droplet size distributions determined by static light scattering were in a nanoscale range and turned out to be similar for all emulsions.

The osmolality was found to be in a physiological range between 290-325 mosmol/kg.

Different hemolytic activity was detected by the hemolytic assay. Emulsions with a mixture of lecithin and sodium oleate as stabilizers caused lysis of red blood cells. Samples containing a mixture of lecithin and poloxamer 188, showed a distinct lower hemolytic activity. The optimized tolerability was found for systems that included lecithin and poloxamer 188 (1:1) and were isotonized with sorbitol.

Conclusion
The study showed that a high emulsifier content of 5 % not necessarily lead to high hemolytic activity of the sample.

References
Thermal stability and aggregation of a MAb and its Fab and Fc fragments

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Introduction
The role of different domains of monoclonal antibodies (MAbs) on aggregation and unfolding and overall stability of the full MAb is of high interest. To understand the thermal behavior of an IgG1 therapeutic MAb and its Fab and Fc fragments, melting profiles and T_m values from differential scanning calorimetry (DSC) and -fluorimetry (DSF) were compared. Additionally, the temperature of aggregation was obtained by turbidity (A_350mn) measurements.

Materials and Methods
After papain digestion, the fragments were purified with ultrafiltration, protein A affinity, and size exclusion chromatography. The purity was confirmed by hydrophobic interaction chromatography. Samples were prepared with phosphate buffers at pH 7.2 and 5.0 +/- 140 mM NaCl. DSC and DSF were performed as described recently [1].

Results and Discussion
Unfolding and aggregation of the MAb and its Fab and Fc fragments strongly depends on the pH and ionic strength of the solution. At pH 7.2, the DSC melting profiles of Fab, Fc, a 2:1 mixture of Fab and Fc (“(Fab)_2Fc”), as well as the full MAb show a perfect overlay. At pH 5.0, the thermal stability of the Fc part is dramatically reduced. In addition, T_m values of both fragments were also lower compared to the values of the domains of the full MAb.

Fig.1: DSC melting profiles at pH 5.0
This letter effect was confirmed with DSF and suggests a mutual stabilization of the full protein at the low pH. Additionally, T_m of (Fab)_2Fc and the individual fragments coincided for DSF, whereas the DSC data suggest that Fab and Fc was even destabilized at pH 5.0 when mixed in solution (Fig.1). The positive net charge at pH 5.0 was responsible for a colloidal stabilization of the protein. At pH 7.2 and when charges are shielded by NaCl, strong aggregation occurred. Furthermore, the temperature of aggregation from turbidity measurements corresponds to the onset temperature of unfolding of certain domains. In combination of these methods with respect to the outcome of the Fab and Fc fragments, a clear picture of the thermal stability of the full MAb was obtained.

References
Rheological study of chitosan/polyol-phosphate systems: influence of the polyol part on the thermo-gelling mechanism

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Introduction
Thermo-sensitive gelling systems, like chitosan (CS) / polyol-phosphate, are high potential candidates for the design of biodegradable \textit{in situ} forming depots [1]. Even if the physicochemical and rheological properties of CS / β-glycerophosphate (β-GP) systems are well known and characterized, the impact of the gelling agents' chemical structure, and notably the polyol group, on the overall gelation behavior, is still not elucidated [2,3]. To further assess the role of the polyol-moiety in the gelation mechanism, a comparative rheological study of different CS-based thermo-gelling systems was performed, using β-GP, glucose 1-phosphate (G1-P), glucose 6-phosphate (G6-P), and inorganic phosphate salt as gelling agents.

Materials and Methods
Preparation of CS/gelling agent solutions: Chilled gelling agent (β-GP, G1-P or G6-P) solution was added to the cold CS solution under stirring. The thermogelling solutions were produced from 1.5 wt% CS and 0.17 to 0.43 mmol/g β-GP, G1-P or G6-P.

Rheological tests: rheological properties of the thermogelling solutions were investigated as a function of angular frequency, temperature and time. For all experiments, the sol/gel transition temperature (T\textsubscript{SG}) or time point (t\textsubscript{SG}) corresponds to the intersection of the curves of the storage modulus G’ (representing the elastic behavior) and the loss modulus G” (reflecting the viscous behavior).

Results and Discussion
The comparison of gelation behavior of CS solution combined with inorganic phosphate salts, β-GP, G1-P or G6-P, indicated that the glucose-phosphate salts had the same ability as the commonly used β-GP to transform CS solutions into thermosensitive systems. G1-P and G6-P neutralized CS solutions while keeping them in the solution state at room temperature, and gelation took place upon moderate heating. Our study suggests that the polyol moiety of the gelling agents is responsible for the thermal sensitivity of the CS solution and that the chemical structure and size of the polyol plays a significant role in controlling the gelation process. It is assumed that polyols create a hydration protective layer around the CS chains, built through weak intermolecular interactions, like hydrogen bounds. A temperature increase destroys this polyol layer and allows the CS chains to interact with each other through stronger hydrophobic bonding, thus inducing gel formation.

Conclusion
This study showed that the size of the gelling agents' polyol part in CS thermogelling systems has an impact on T\textsubscript{SG} and on the kinetics of gel formation, and finally on the gel’s strength.

References
Hydrophilic nanoparticles for hydrophilic macromolecular drugs

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Introduction
Among the approaches for the delivery of macromolecules to the desired site of the body, nanoparticles have shown great potential. Macromolecules are typically encapsulated or entrapped within the nanoparticle matrix, depending upon the preparation method. The smaller size of nanoparticles favors their uptake into the cells, compared to bigger size delivery systems [1]. Therefore, nanoparticles have gained considerable attention as delivery vehicle for macromolecules. However, most of these systems utilize hydrophobic polymers, which in most cases leads to inactivation of macromolecular drugs such as proteins [2].

Gelatin is a biocompatible and biodegradable biopolymer. Its hydrophilicity offers good compatibility with hydrophilic macromolecular drugs, resulting in higher probability to entrap macromolecules. Which is why, gelatin is receiving interest as a nonmaterial for the delivery of macromolecules [3].

Materials and Methods
Gelatin contains both anionic and cationic groups in its structure. Upon contact with non-solvent, an intermolecular charge neutralization of gelatin occurs, hence an inter-particular aggregation occurs. Besides this, crosslinking is applied to improve the mechanical properties of gelatin, which additionally favors inter-particular crosslinks. In order to prevent intra-particle aggregation we used stabilizer in the non-solvent.

A straightforward preparation technique, based on the addition of an aqueous gelatin solution to ethanolic solution of stabilizer, was established.

Gelatin nanoparticles are formed immediately when water diffuses to the ethanol, which are then crosslinked with glutaraldehyde in order to obtain stable nanoparticles. Several parameters were investigated, such as concentration of stabilizer, surfactant type, solvent/non-solvent ratio, and gelatin concentration in the solvent phase.

Results and Discussion
Stable nanoparticles were obtained with Poloxamer 407 or Poloxamer 188 as stabilizers. FITC-dextran as hydrophilic model drug could be entrapped in the nanoparticles. The load showed a burst release up to 20% within the first hour, with no additional release over 5 days. However, trypsin digestion released the remaining drug completely [4].

Conclusion
Gelatin nanoparticles with sizes of about 250nm and 280nm could be obtained with ethanol and methanol as non-solvent respectively. The particles remained stable throughout the crosslinking process. Furthermore, the potential for macromolecule incorporation could be demonstrated.

References
Characterization of Interactions between Silk Fibroin and Protamine

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Introduction
Silk fibroin (SF) is an emerging biomaterial for protein-drug delivery systems. SF contains hydrophobic elements flanked by charged moieties. Consequently, electrostatic binding as well as hydrophobic interaction have been linked to SF/protein-drug binding.

Materials and Methods
Enthalpy changes during interaction of SF and the positively charged model protein-drug protamine were followed by isothermal titration calorimetry (ITC) and parallel to ζ-potential and turbidity measurement, respectively. This data was correlated to colloid kinetics of SF and protamine alone as well as of mixtures of increasing molar ratios. The mechanism of SF-protamine interaction was studied in a histidine buffer or NaCl.

Results and discussion
An initial endothermic response was observed at low protamine/SF ratio, potentially linked to structural changes and as described before. The intensity of the heat signal was impacted by the solution composition. Increasing the protamine/SF ratio was parallel to a gradual shift from endothermic to exothermic signals, linked to counter ion and water release as a result of increasing occupation of SF binding sites for protamine. Ongoing studies link this observation to protein-drug loading efficiency and release kinetics, respectively, and to meet demands of pharmaceutical quality – essential to deploy SF as a future protein-drug delivery system.

References
Ultra-small scale for hot extrusion nanoparticle production

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Introduction
Hot high pressure homogenization is typically used to produce nano-sized lipid carriers. This technique is approved and suitable for both lab and large scale production. Nevertheless, in early drug development a limitation of active compound yield is a crucial cause for a preliminary production. Therefore, the development of a production method which needs a very small amount of sample is required. In this study, a small-volume extruder, normally used for liposome production, was used for the production of nano-sized lipid carriers [1].

Materials and Methods
Lipid phase and water phase were melt separately. Then water phase was mixed into lipid phase and pre-mixed by magnetic stirrer. After that pre-emulsions were pressed through the 100 nm pore size membrane using an extruder (LiposoFast-Basic, Avestin, Canada) up to 29 cycles. Each batch size was 0.5 ml. Samples were drawn at different cycles during the production. Particle size, polydispersity index (PI) and zeta potential were analyzed by photon correlation spectroscopy.

Results and Discussion
For the nanoemulsion, the smallest droplet size, obtained from 11 extrusion cycles, was 440 nm with PI higher than 0.2. And zeta potential is around -70 mV. However, more cycles of extrusion did not decrease the droplet size. Solid lipid nanoparticles (SLN) produced by the extruder obtained a size of 205 nm and 180 nm after 21 cycles and 29 cycles respectively. And the PI was lower than 0.2. The zeta potential of both formulations of SLN was around -60 mV. The production of nanostructured lipid carriers (NLC) led to a particle size of about 190 nm after 21 cycles. Further cycles did not lead to a size reduction. Moreover, both SLN and NLC formulation were stable within 1 month.

Conclusion
SLN and NLC, produced by the ultra-small scale extruder, reached a suitable nanosize (179 nm and 190 nm respectively). This method is not expensive, easy to use and required a very small amount of drug. However, the method was not suitable for the production of nanoemulsion which required a high temperature during the production. Future work will compare the results obtained from the extruder with results obtained by high pressure homogenization.

Protein mobility in Diels-Alder Hydrogels

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Introduction

The incorporation of proteins into hydrogels is an excellent method to increase their stability and prolong their biological half-life [1]. The protein release can be restricted by the hydrogel network mesh size. While the mesh size slowly increases during gel degradation, the protein is gradually released over time (Figure 1). Here, we present the mobility of different model proteins in poly(ethylene glycol) (PEG) based hydrogel networks.

Materials and Methods

For hydrogel preparation, branched PEG was cross-linked by Diels-Alder reactions in the presence of different proteins. Directly after cross-linking, the mobility of FITC-lysozyme, FITC-BSA and FITC-γ-globulin was determined by fluorescence recovery after photobleaching (FRAP) experiments [2].

Results and Discussion

FRAP experiments showed different mobility of the used proteins depending on their size. FITC-lysozyme ($d_H = 3.8$ nm) showed a mobile fraction ($k$) of 0.48 and a diffusion coefficient ($D$) of 8.42 µm²/s. FITC-BSA ($d_H = 7.1$ nm) had a considerably lower mobility ($k = 0.68$, $D = 0.22$ µm²/s). FITC-γ-globulin ($d_H = 14.2$ nm) was completely immobile ($k = 0.04$). With regard to the calculated network mesh size [3], we expect lysozyme to be released from the non-degraded hydrogels. The release of BSA and γ-globulin is probably delayed until gel degradation sufficiently proceeded.

Conclusion

Our experiments showed a different mobility of the tested model proteins within the non-degraded hydrogel network. In the next step, we will follow the increasing protein mobility over hydrogel degradation time. The obtained data will be compared to concomitant release experiments.

References


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Novel starch based biodegradable polymers for gene delivery

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Introduction

Cationic polymers are attractive nonviral gene delivery vectors since they can condense anionic polynucleotides into nano-scaled, charge stabilized polyplexes. We synthesized graft-polymers composed from starch backbone and low molecular weight (MW) polyethylene-imine (PEI, 800 Da) side-chains. In comparison to the transfection standard of 25 kDa bPEI this new polymers should allow to reduce toxicity, retain transfection efficiency and achieve biodegradability. By adjusting a) the PEI modification amount and b) MW of starch, we obtained a variety of polymers with different characteristics (Fig. 1). Here we proof the high potential of some synthesized starch-g-PEI polymers as a gene delivery vector.

Materials and Methods

Partially hydrolyzed potato starch (kind gift of AVEBE, Veedam, NL) was used in all experiments. PEI, 800Da and all other materials were purchased from Sigma-Aldrich. First starch was oxidized by TEMPO mediated method¹ and separated into three MW fractions. The conjugation reaction between starch and PEI was done using DMTMM². The synthesized starch-g-PEI polymers were characterized with ¹H-NMR, GPC and subjected to α-amylase degradation assay. Polyplex formation of the various starch-g-PEI polymers with luciferase-encoding pDNA was assessed. Finally cytotoxicity (LDH/MTT) and transfection efficiency in A549 cells was measured.

Results and Discussion

The polymer synthesis allowed controlled modification with PEI of 20, 30 and 40 wt%, which was verified by ¹H-NMR and GPC. PEI modification of 30 wt% did not alter significantly the speed of degradation by α-amylase. Both, polyplex stability and transfection efficiency, was highly influenced by the used polymer. The best transfection efficiency was observed with starch-g-PEI 30 wt% modification and middle or large MW starch backbones. The cytotoxicity based on IC₅₀ of the best polymer was three to four times lower than that of PEI 25 kDa.

Conclusion

We synthesized a biodegradable cationic starch-g-PEI polymer. The careful balance of suitable amount of modification and polymer MW allows a tuning of desired properties for a successful gene delivery vector.

References

Solid State Effects on The Particle Size Reduction of Resveratrol

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Introduction

H 42 is a two-step particle size reduction method to produce drug nanocrystals. Rapid drying from organic solutions (e.g., spray-drying, rotavapor, etc.) is used as first step to produce solvent-free, fragile drug powders which are subsequently processed by aqueous high pressure homogenization (HPH) to nanosuspensions.

Previous studies with glibenclamide indicated improved size reduction effectiveness for amorphous drugs. This led to the assumption that amorphous solids could be more fragile compared to crystalline solids.

In order to further identify the effect reduced crystallinity on the particle size reduction effectiveness, various unmodified and spray-dried resveratrol samples have been processed with HPH and analyzed for their particle size and solid state before and after HPH.

Materials and Methods

Resveratrol (RVT) and sodium cholate (SC) were co-spray-dried. The ratios (RVT: SC) in ethanol were 1:0.75, 1:0.146 and 1:0. RVT and HPMC (1:2, 2:1 and 1:1) solutions were dried via rotavapor. Raw RVT was also modified by melting and quench cooling. All modified samples (SD, rotavapor and quench cooling) and raw RVT were nanosized by a micron lab 40 homogenizer. DSC and PXRD were used to characterize solid states before and after HPH.

Results and Discussion

The spray-dried powder (1:0.75 RVT/SC) was predominately amorphous. Amorphous RVT was also found as resulting from the rotavapor process (1:2 RVT/HPMC). Quench cooling could not reduce the crystallinity of RVT.

In contrast to our starting hypothesis the use of amorphous RVT did not result in smaller particles as compared to crystalline RVT after HPH. However, modification of RVT by means of SD was beneficial and led to the smallest particle sizes.

Interestingly, DSC analysis of nanosuspensions from unmodified RVT suggested a reduced crystallinity. This was in contrast to the results of PXRD. The hypothesis was that an amorphous conversion occurred during the DSC measurements as result of relatively high surfactant or polymer concentrations. Therefore nanosuspensions have been filtered and washed before further measurements. DSC analysis of the dried residues then only showed one melting endothermic peak and no glass transition.

Conclusion

In case of RVT amorphous drugs showed no benefit on the particle size reduction effectiveness. Ultra small nanoparticles were achieved when predominantly crystalline spray dried RVT was used. In addition, it is strongly recommended to remove surfactants or polymers from the nanosuspensions before performing any solid state analysis.

References

Ternary liposome-polyethylenimine complexes for pDNA and siRNA delivery

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Therapeutic nucleic acids like pDNA for gene therapy or small interfering RNA (siRNA) for specific gene knockdown (RNA interference, RNAi) have shown promise for the treatment of various human diseases including cancer, infectious diseases or genetic disorders. The major challenge is the safe and efficient delivery of pDNA or siRNA. The inability of naked nucleic acids to penetrate negatively charged cell membranes and reach their target cells, and their insufficient stability requires the development of adequate carriers.

Cationic polymers and lipids are the most common synthetic vectors to address this issue. They are able to condense nucleic acids into nanosized complexes by electrostatic interactions, thus protecting their payload from degradation and promoting cellular uptake. Polyethylenimines (PEI), the gold standard of cationic polymers used as non-viral vectors, are available in branched or linear structures in a wide range of molecular weights. A drawback of PEI-based complexes is the strong cationic charge which can lead to cytotoxicity, complex aggregation, and non-specific interactions with blood and cell components.

We have developed a novel, biocompatible and physicochemically more stable PEI-based delivery platform for in vitro and in vivo use. By non-covalent modifications of PEI polyplexes with phospholipid liposomes, we established ternary complexes (so-called ‘lipopolyplexes’). For polyplex formation, three different PEIs are employed: the non-commercial 4 - 10 kDa branched PEI F25-LMW as well as two commercially available 25 kDa linear PEIs with different degrees of deacylation.

Liposomes used in this study mainly comprise dipalmitoylphosphatidylcholine (DPPC) and various colipids with different physicochemical properties, such as the cationic lipid (DOTAP) or the “stealth” PEG-lipid DPPEmPEG5k.

Liposome-PEI complexes are characterized in terms of composition and various modes of preparation. Their biological evaluation reveals decreased cytotoxicity and highly efficient in vitro delivery of pDNA and siRNA. Zeta potential measurements indicate reduced surface potentials compared to the “parent” PEI polyplexes. For size measurements, we compare dynamic light scattering with the relatively new “nanoparticle tracking” technique (NTA). Moreover, we show that some PEI-based lipopolyplexes allow prolonged storage and display increased stability in the presence of serum proteins. We conclude that liposome-PEI complexes may offer innovative avenues for the application of therapeutic nucleic acids.

References
A controlled modification model for construction of functionalized silk fibroin for biomedical applications

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Introduction
Silk fibroin (SF) has been hotly focusing on by scientists for a long time due to its good biocompatibility, unique mechanical properties and opportunities for genetic tailoring of structure. However, the lack of cell response seems to block its further biomedical application. It is well understood that a variety of factors govern the interaction between cells and scaffolds, such as cell adhesion, proliferation, differentiation, migration and sometimes even survival. Therefore, modification on specific site of SF molecule will potentially expand its clinical use [1].

Materials and Methods
Three steps are included in this SF modification process: (1) Extraction and purification of SF solution; (2) Preparation of azido-SF through diazo-coupling reaction; (3) Specific binding alkyne-terminated molecules to azido-SF with controlled mole ratios through CuAAC process. Uv-vis and FTIR were used to characterize the assembly efficiency between model molecules and SF.

Results and discussion
Uv-vis results indicated that tyrosine group of SF can be modified with consequent rate from 1.03 (mol) % to 70.3 (mol) % through controlling the addition amount of diazo-reagent into the reaction system. The stable azido-SF solution could be obtained when the modification rate was lower than 5 (mol) % and higher than 70 (mol)% with respect to the amount of tyrosine from SF, while the gel could form easily when the modification rate was ranged between 10 (mol)% and 60 (mol)%. FTIR spectra of diazo-SF film indicated that the β-sheet formation could be induced by the modification rate over 3 (mol)% with respect to the tyrosine group in each SF molecule. Two fluorescent dyes with terminal acetylene group (Acetylene-Fluor 488 and Acetylene-Flour 585) were employed to investigate the specific modification efficiency on the azido-SF through CuAAC process. The results showed that the linking ratio of two dyes with azido-SF strongly depended on their mole ratio in the reaction system.

Conclusions
Controlled assembly of azido-group into SF molecules can be achieved through diazo-coupling process that benefits the quantative modification of SF through CuAAC process. This process can be used for the preparation of functionalized SF which can be further used as drug delivery device for biomedical purpose.

References
CoQ_{10}-loaded ultrasmall lipid nanocarriers (usNLC) for the deeper penetration

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Introduction
Coenzyme Q\textsubscript{10} (CoQ\textsubscript{10}) is a lipophilic compound with a strong antioxidant capacity, making it an interesting drug for treating oxidative stress. However, the bioavailability upon dermal application is very limited. Solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC) were invented since 20 years ago and proved to be an innovative lipophilic drug delivery system for many applications routes (e.g. oral, dermal, ocular, intravenous) [1]. Using hot high pressure homogenization, the size of NLC is typically in the range of 150 - 300 nm. In previous studies, the particle size is a crucial parameter for the bioavailability of these carriers, e.g. particles < 100 nm can be taken up more efficiently [2]. Therefore, the aim of this study was to evaluate the physicochemical properties, the \textit{in vitro} release profile and \textit{ex vivo} penetration studies of CoQ\textsubscript{10}-loaded ultrasmall lipid nanocarriers (usNLC) with particle size below 100 nm compared to classical carriers, i.e. nanostructured lipid carriers (NLC), and the traditional carriers, i.e. a nanoemulsion (NE).

Materials and Methods
5\% w/w of CoQ\textsubscript{10}-loaded NLC, NE and usNLC were produced by hot high pressure homogenization technique.

Results and Discussion
The particle size of NLC was approximately 210 nm, the similar particle size of NE and usNLC was about 85 nm by PCS analysis. All samples possessed similar a good physicochemical stability over a period of 3 months. The highest accumulated amount of CoQ\textsubscript{10} for the usNLC was shown \textit{in vitro} release studies using static Franz diffusion cells and HPLC analysis, followed by the NE and NLC, respectively. In \textit{ex vivo} stripping test, only CoQ\textsubscript{10}-loaded usNLC was determined in the five strips indicating the best lipid-based formulation for the skin penetration enhancement compared to NE and NLC.

References
Spray-dried Alginate Microspheres as Controlled Release System for Pulmonary Protein Delivery

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Introduction
Alginate, a natural, linear polysaccharide, gains increasing attention as biopolymer matrix for controlled protein delivery. As it forms water insoluble hydrogels after cross-linking by bivalent cations like Ca\(^{2+}\) or Zn\(^{2+}\), it offers a relatively inert aqueous environment within the matrix and is biodegradable under normal physiological conditions, alginate is well suitable for the encapsulation of bioactive proteins [1], [2].

Materials and Methods
To prepare a controlled release system for pulmonary protein delivery, microparticles were produced by spray-drying of aqueous solutions of alginate, Zn(NH\(_3\))\(_4\)SO\(_4\) and the model protein bovine serum albumin (BSA) [2]. Due to evaporation of NH\(_3\) during spray-drying, Zn\(^{2+}\)-ions were released, resulting in cross-linking of alginate chains. Hence, the protein is entrapped into the cross-linked alginate network during spray-drying with high encapsulation efficiency. The process was adapted to the Nano Spray-Dryer B-90 (Büchi Labortechnik AG, Flawil, Switzerland), which provides a more narrow particle size distribution than standard spray-drying setups and furthermore is suitable for reproducible processing of small amounts of material [3]. The alginate type and the size of the perforation of vibrating mesh nebulizer were varied and the effect on particle size and drug release was analyzed. Three different alginates of various viscosities and proportion of guluronic and mannuronic acid residues were examined. The particles obtained were characterized using dynamic light scattering as well as SEM regarding size and surface morphology.

Results
The particles produced by the Nano Spray Dryer are clearly smaller than particles produced by a Mini Spray Dryer [2]. Alginate-BSA-particles cross-linked by Zinc-ions are collapsed and possess a rough wrinkled surface while particles consisting only of alginate are spherical and have a very smooth surface.

References
Diels-Alder Hydrogels with Defined Mesh Size for Controlled Drug Delivery

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Introduction
Due to their customizable properties, hydrogels are an interesting alternative to conventional drug delivery systems. The release rates of incorporated drug molecules can be controlled by the hydrogel network mesh size. Here, we present our studies on poly(ethylene glycol) (PEG) based hydrogels cross-linked by Diels-Alder reactions. The network mesh size of the synthesized hydrogels was calculated and the obtained results were used to estimate the release rates of incorporated molecules.

Materials and Methods
PEGs with two different branching factors and two different molecular weights (4armPEG10k, 8armPEG10k and 8armPEG20k) were used for gel preparation. The polymers were modified with furyl and maleimide groups to yield two complementary macromonomers according to previously established protocols \([1]\). These were dissolved in water, mixed and allowed to gel. Afterwards, the gels were swollen in water and the network mesh size was determined \([2]\).

Results and Discussion
Depending on the concentration, branching factor and molecular weight of the used PEG, hydrogels with different properties were formed. The determined mesh sizes varied from 44.0 ± 17.3 nm (5 % 4armPEG10k) to 5.4 ± 0.4 nm (15 % 8armPEG10k) (Table 1). The determined values can be used to estimate the release rates of incorporated drug molecules. Small molecules are expected to be released from all hydrogels since their dimensions are well below the determined mesh sizes. In contrast to that, the mobility of larger macromolecules, such as peptides or proteins, is expected to be restricted within the hydrogels. The release rates will depend on the hydrodynamic radius of the incorporated molecule and the network mesh size of the hydrogel carrier.

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<td>8armPEG20k</td>
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Conclusion
The Diels-Alder reaction is a useful cross-linking mechanism and allows for the synthesis of hydrogels with defined mesh size. Our experiments showed that the network mesh size depends on the concentration, branching factor and molecular weight of the used macromonomers. The developed hydrogels are suitable to control the release of incorporated macromolecules.

References

Financial support from DFG, grant GO565/16-1 is gratefully acknowledged.
Suitability of Eudragit® polymers for the preparation of dry Pickering emulsions

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Introduction
Solid stabilised emulsions, also known as Pickering emulsions (PE), are extremely stable. Therefore, they can be used to produce dry emulsions [1,2].

For oral use, PE are preferably used as dry emulsions which can be obtained by drying in a fluid bed granulation process [2]. PE serve as liquid binder. For this purpose it is crucial to add a polymeric binder to the PE. Until now, only water soluble polymers, e.g. HPMC, have been investigated successfully [2].

The aim of this study was to substitute HPMC by several Eudragit® polymers which are insoluble and swell pH independently. Thus, an oral drug delivery system with modified release characteristic can be obtained from dry PE. In a first step, we evaluated the compatibility of PE with aqueous dispersions of these polymers.

Materials and Methods
Miglyol 812® (Sasol, D-Hamburg), Eusolex® T-2000 (Merck, D-Darmstadt), Eudragit® RL 30 D, Eudragit® RS 30 D, Eudragit® NE 30 D (Evonik Industries AG, D-Essen) and purified water.

PE consisted of 40 % Miglyol®, 6 % Eusolex® and 54 % purified water. PE was produced in a Becomix RW 2,5 (Mischtechnik A. Berents GmbH, D-Stuhr). One part of polymer dispersion (10 % aqueous dispersion) was added to one part of PE.

Flocculation was evaluated microscopically with a Zetasizer® Nano-ZS (Malvern Instruments™ Ltd, UK-Worcestershire).

Results and Discussion
Fig. 1 compares the droplet size distribution of PE before and after adding polymer dispersions. The droplet size distribution of PE with 10% Eudragit® NE is shifted to higher values in comparison with PE without polymer dispersion due to massive flocculation. After addition of cationic Eudragit® RS, droplet size distribution remained almost unchanged with only very weak flocculation. Eudragit® RL did not induce any flocculation. This indicates that electrostatic repulsion of the cationic polymers and the positively charged titanium dioxide (at pH 2 - 6) allows to obtain stable PE. With mixtures of Eudragit® NE and Eudragit® RL or Eudragit® RS we could demonstrate that it is possible to overcome the incompatibility with Eudragit® NE. Again, the limiting factor is the amount of cationic groups in the polymer mixtures.

Figure 1: Droplet size distribution of PE and PE with polymer dispersions; n = 3; error bars = SD

Conclusions
Functional polymer dispersions with ionic groups are compatible with Eusolex® stabilised PE. Emulsion stability depends largely on the amount of ionic groups when mixtures of ionic and nonionic polymer dispersions are used.

This can make them useful to get dry emulsions with modified release characteristics.

References
Optimization of the precipitation process to generate smaller size drug nanocrystals

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Introduction

In 2001, Kipp et al. combined precipitation with high pressure homogenization process to generate smaller size drug nanocrystal. However, later we demonstrated that reduction of the time difference between precipitation and high pressure homogenization could significantly reduce the particle size compared to the previous process. It was realized that there was further possibility to reduce the particle size. The purpose of this work was to optimize the solvent type, internal stabilizer type and its content to further reduce the particle size. Resveratrol was used as a model compound due to its poor water solubility and sufficient solubility in different organic solvents.

Materials and Methods

Resveratrol was purchased from E.denk FineChemie GmbH, Germany. The solvent phase (S) was prepared by solubilizing the drug in one of the 7 water miscible organic solvents such as alcohols (Ethanol, Methanol, Isopropanol), acetone, tetrahydrofuran (THF), dimethylformamide (DMF), dimethylsulfoxide (DMSO) and binary compositions of a few solvents. An aqueous solution of sodium dodecyl sulphate (0.2% w/w) was mixed with 0.5% polyvinylpyrrolidone (K40) to constitute the anti-solvent phase (AS). Using a needle and a pump system, the S-phase was injected in the AS phase just before the homogenization zone in an Emulsiflex C5 (Avestin Europe GmbH) operated at 1000-1200 bar. The process was optimized with addition of a range of stabilizers having HLB values from 1 to 29 in the organic phase. Particle sizes of the drug nanocrystals were measured by laser diffraction and photon correlation spectroscopy. Particles were also observed by light and electron microscopy.

Results and Discussion

Based on the particle size data, DMSO and a binary combination of DMSO and Acetone generated smaller size particles compared to the other solvents. These two samples had D(0.9) size between 897 nm-929 nm and D(0.5) size between 147-157 nm with 10% particles bellow 70 nm in size.

When Span 20 was added to the S-phase the D(0.9) size was further reduced to 560 nm. However, no significant changes were observed for the D(0.5) value. The Z-average of this sample was 245.8 nm.

The particle size was also affected by the resveratrol:Span 20 ratio. Here 1:3 was identified as the optimum ratio.

Stabilizer with HLB value of 10±2 was found to be the best in this experiment. Scanning electron microscopy revealed that most of the larger particles were in fact aggregates of smaller particles having a size of about 40-50 nm.

References

Production & stability of dermal glycyrrhetinic acid nanosuspension

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Introduction

Glycyrrhetinic acid (GHA) is a poorly water soluble terpenoid having excellent antioxidant activity and therefore thought to have beneficial effect on skin. However, the beneficial activity of the compound is not realized because of its poor skin penetration property. Nanosuspension has proved to be a delivery platform to improve the skin penetration of poorly water soluble compounds [1]. In this study, we wanted to demonstrate whether it is possible to formulate GHA in a stable nanosuspension formulation.

Materials and Methods

GHA [5% (w/w)] was dispersed in 1% (w/w) aqueous solution of Plantacare 2000 UP to prepare the coarse suspension. GHA Nanosuspension was prepared by a combinative process [2]. The coarse suspension was homogenized by pearl mill (PM, Bühler PML-2, Bühler AG, Switzerland) for 150 min followed by high pressure homogenization (HPH, Micron LAB 40, APV Deutschland GmbH) for 20 cycles. The physical parameters of the GHA nanosuspension were measured by photon correlation spectroscopy (PCS, Zetasizer Nano ZS, Malvern Instruments, UK), laser diffractometry (LD, Mastersizer 2000, Malvern Instruments, UK), light microscopy and powder X-ray diffraction (PXRD, Philips X-ray Generator PW 1830, Amedo, The Netherlands). The samples were stored at 4°C, 25°C and 40°C for stability assessment. Saturation solubility of the drug from the coarse suspension and the nanosuspension were determined at 25°C in water, after equilibration for 7 days.

Results & Discussion:

The GHA nanosuspension had a mean z-average of 155 nm and polydispersity index of 0.177, when measured by PCS. The z-average was well correlated with the d (0.5) value from LD, i.e. 121 nm. The zeta potential value of -31.5 mV in original dispersion medium (1% Plantacare 2000 UP solution), indicated a reasonable physical stability of the nanosuspension. The formulation was stable for 1.5 years in terms of its particle size and crystallinity. The equilibrium solubility (7 days) of the drug from the coarse suspension and the nanosuspension were 4 µg/ml and 80 µg/ml, respectively.

Second generation smartCrystal® technology has been proved to be successful to generate smaller sized GHA nanosuspension, which is stable for almost 1.5 years. The smaller size GHA nanosuspension has improved saturation solubility in water. GHA nanosuspension formulations can be further admixed to cosmetic or pharmaceutical bases for dermal applications.

References

Novel filamentous Carrier Systems based on biodegradable Hydrogels

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Introduction

Design of a prolonged release system is one of the topics of highest interest in the field of pharmaceutical technology, particularly in the micro- and nanometer size range. A prerequisite for an effective sustained release system is an adequate residence time in the target region, governed by the clearance. Literature indicates a strong impact of shape on phagocytosis, an essential mechanism for clearance [1]. Among other morphologies, fibers or rods drastically reduce the likelihood of ingestion and therefore extend the residence time.

Hydrophilic carrier systems such as gelatine and alginate are of interest forming gel-like particles, permitting to load hydrophilic compounds to a high extend. A carrier based on gelatine allows delivering hydrophilic and macromolecular drugs, offering intriguing properties such as biocompatibility, biodegradability, low antigenicity, low cost, and numerous available functional groups for modification. The natural polysaccharide alginate forms gels in the presence of bivalent ions such as calcium. The integrity of the gel scaffold depends on the concentration of the respective ion. Over time the Ca²⁺ conc. will be depleted by diffusion. Additionally, the incorporation of nanoparticulate systems within the hydrogel fibers is possible [2].

Materials and Methods

A template-assisted approach is applied in order to form fibers in high fidelity. Membranes with uniform cylindrical pores serve as templates for the fibers. This void space of the template is filled with the liquid precursor of the hydrogel. Being in the sol state, the hydrogel can access and fill the pores. Depending on the hydrogel, either a CaCl₂ solution (alginate), or the crosslinker glutaraldehyde/GTA (gelatin) is added in order to perform solidification.

Results and Conclusion

The geometry of the fibers can be customized by the selection from a broad spectrum of pore sizes of the membranes. Fibers in various diameters ranging from 0.4 to 5 µm could be successfully generated. Results of the first loading experiments will be presented.

References


Surface Dependent Plasma Protein Binding, Biocompatibility and Thrombogenic Potential of Superparamagnetic Iron Oxide Nanoparticles

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Superparamagnetic iron oxide nanoparticles (SPIIONs) are used as contrast agents for diagnostic purposes in magnetic resonance tomography (MRT) \cite{1}. Since they are commonly intended for systemic administration, investigation of cytotoxicity and hemocompatibility are required.

Several non-charged, anionic and cationic polymer coated superparamagnetic iron oxide nanoparticles were characterized regarding size and charge and analyzed \textit{in vitro} and \textit{in vivo} regarding their interaction with plasma proteins, cyto- and hemocompatibility. Binding profiles with human plasma and single plasma proteins were analyzed quantitatively using bicinchoninic acid assay and SDS-PAGE. Erythrocyte aggregation and hemolysis were quantified spectrophotometrically in the presence and absence of plasma proteins. Additionally, the chick area vasculosa (CAV) was used as \textit{in vivo} hen’s egg model. SPIIONs were injected intravenously, and CAV was examined for vascular lysis, hemorrhage, thrombi and exitus \cite{2}.

All particles showed hydrodynamic diameters of approximately 150 nm. With an increase of zeta potential, the binding of plasma proteins increased with the ranking neutral \textless anionic \textless cationic surface. As a trend, all particles were preferentially coated by fibrinogen, followed by human serum albumin and \textgammaglobulin. Only for cationic SPIIONs cyto- and hemotoxicity increased with higher charge density and, could be reduced in presence of plasma proteins. In the CAV model all particles caused time dependent toxic effects over up to 24 h. Thrombotic events could be observed for all types of particles with a higher lethality for the cationic particles with high surface charge density. The particles did only negligible influence vascular lysis and hemorrhage. The effects of SPIIONs on CAV correlated well with the results of cyto- and hemocompatibility testing \textit{in vitro}.

References


Acknowledgements

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Improvement of Kinetic Solubility of Tailor-made Quercetin Nanocrystals

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Introduction
Poorly soluble flavonoid quercetin has potent antioxidative effect [1]. To make its application more feasible, nanocrystals were produced and its kinetic solubility was investigated.

Materials and Methods
Quercetin anhydrous was processed into nanocrystals by high pressure homogenization (HPH) [2]. Poloxamer 188 was chosen as stabilizer. Nanocrystals with different sizes were obtained by collecting samples after certain homogenizing cycles. All samples were analyzed by photon correlation spectroscopy (PCS) and by laser diffraction (LD). For the determination of the kinetic solubility an overweight of each sample was dispersed in water and 0.2% Poloxamer 188 solution respectively, followed by incubation under 25°C and 100 rpm shaking. Samples were drawn after 1h, 6h, 1d, 2d, and 3d. After filtration (0.2 µm) and ultracentrifugation (16000g, 1h), the supernatant was analyzed by HPLC.

Results and Discussion
Quercetin nanocrystals with a series sizes were successfully prepared (from 197 nm to 1061 nm) via adjusting the pressure and cycles of HPH. Energy input (pressure) and processing time (cycle number) are the main factors for determining the size of nanocrystals. Fig. 1 shows the results of kinetic solubility of nanocrystals. Smaller sizes presented higher solubility. Due to smaller size, particle surface has greater curvature, thus possesses higher dissolution pressure [3]. The increase of curvature is exponential, thereby the finest one (G with 197 nm) showed a significant solubility increase compared to the others. It has to be noticed that the solubility at 1h was the highest one in each sample. Nanocrystals can be dissolved very fast and form a supersaturated environment for large crystals. This leads to recrystallization over time.

Figure 1 Profile of kinetic solubility

Conclusion
An almost 10-fold increase in kinetic solubility was found when quercetin was transferred into nanocrystals with a size below 200nm.

Reference
Rutin Nanocrystals: Production, Antioxidative Capacity, and Neuroprotection

Chen, Run1, Sauer, Mark3, Durand, Philippe2, Müller, Rainer H.1, Schäfer, Karl-Herbert3, Prost, Michel2, Keck, Cornelia M.1,3,

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Introduction

Antioxidative effect and neuroprotective effect of Rutin has been widely proved [1, 2]. The aim of this work is to investigate if transferring bulk powder into nanocrystals can significantly increase its performance due to enhanced solubility and cell uptaking.

Materials and Methods

Rutin nanocrystals were obtained by high pressure homogenization (HPH) combined with different surfactants (Poloxamer 188, Plantacare 2000 UP and Tween 80). Particle size was analyzed by photon correlation spectroscopy (PCS) and by laser diffraction (LD). Obtained rutin nanocrystals with different formulations and sizes were investigated by KRL Test (Spiral patent) which detects the free radical-induced hemolysis of whole blood and erythrocytes. Antioxidative capacity was given as % increase of control blood half-hemolysis time. Neuroprotection effect was determined by behavior of spinal ganglion cell in cell culture in presence of Amyloid with or without rutin nanocrystals. Behavior of spinal ganglion cell was observed by luminescence microscope with cell stained by RT 97-c + GAM 488, GFAP +GAR 546, and DAPI.

Results and Discussion

Rutin nanocrystals with different formulations (concentrations from 2% to 5%) and sizes were successfully obtained, which had size range from 271 nm to 789 nm. Results from KRL Test implied a significant increase (up to 250%) of antioxidative capacity on rutin nanocrystals compared to the respective bulk powder suspension. Nanosizing could increase the kinetic solubility and possibility of endocytosis, thus enhancing the absorption [3]. The intensified antioxidant capacity was reflected on the increased half-hemolysis time. Investigation on neuroprotective effect of rutin nanocrystals displayed a positive image under luminescence microscope. The spinal ganglion cell with addition of rutin nanocrystals showed a considerably higher viability in presence of Amyloid even still could not reach the blank control. Further study should continue on amplifying the protective effect.

Conclusion

Rutin nanocrystals with different sizes were successfully produced and were shown to possess a neuroprotective effect and a higher antioxidative capacity than coarse powder.

Reference

Surfactants for the formulation of NLC: Effects on size and physical stability

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Introduction
Nanostructured lipid carriers (NLC) are carrier systems for improved delivery of actives [1]. The efficacy is typically tested in cell culture tests. These media typically contain electrolytes, which can lead to a reduction of the zeta potential and thus to agglomeration of the particles [2]. The aim of this study was to develop/improve a NLC formulation which is physically stable in cell culture medium.

Materials and Methods
NLC were coated with various surfactants via dispersing them in either non-ionic or ionic surfactant solutions. NLC dispersed in water were used as control. Each sample obtained was then dispersed in cell culture medium and incubated for 1h. Particle size analysis and zeta potential measurements were performed prior to and after the incubation.

Results and Discussion
Uncoated NLC were not stable in cell culture medium, i.e. within 1h the size increased from 40nm to 72nm. When particles were coated with 2% TPGS, Tween, PLX 407 or PVP40T a stabilization effect was observed, i.e. no or little changes in size were observed. Less stabilization was observed for the particles coated with only 1% stabilizer. Coating the particles with Poloxamer 188 and SDS were not efficient for physical stabilization.

Zeta potential (ZP) analysis was in line with the results obtained from size analysis. Uncoated NLC analysed in water possessed a ZP of -30mV. Coating with non-ionic stabilizers led to a decrease in ZP, indicating the adsorption of the stabilizers onto the surface of the particles. The ZP further decreased with increasing surfactant concentration, i.e. thicker stabilizer layers were formed. The highest decreases in ZP were found for the most stabilizing surfactants TPGS, Tween, PLX 407 and PVP40T coated with 2% surfactant solution. Coating with SDS led to an increase in ZP to -70mV. However, upon addition of the cell culture medium it dropped down to -9mV, indicating insufficient stabilization of NLC by using ionic surfactants.

Conclusion
Coating NLC with non-ionic surfactant can improve their physical stability in cell culture medium. Zeta potential analysis is a suitable method to predict the stabilization efficacy of the surfactant.

References
Introduction
Fibroblast growth factor-binding protein 1 (FGF-BP1) is an interesting polypeptide for the treatment of impaired wound healing and tumor-dependent angiogenesis[1]. By binding non-covalently and irreversibly to at least FGFs 1, 2, 7, 10 and 22, it releases these growth factors from the extracellular matrix (ECM) and protects them from proteolytic degradation[2]. Thus one peptide can alter the activities of multiple factors which compose the complex pathway(s) of angiogenesis. In order to investigate this controlled release mechanism we first established a suitable expression and purification procedure of the transport protein.

Materials and Methods
Recombinant murine FGF-BP1 with N-terminal Fc-tag and a signal sequence for secretion was expressed in HEK 293 F cells using the PEI transfection system. Transfected cell supernatant was loaded on Protein A columns using an ÄKTA Purifier system.

Results and discussion
Western analysis and SDS-PAGE analysis of the recombinant protein confirmed the suitability of the expression and purification procedure.

References

Controlled protein delivery from electrospun non-wovens: Novel Combination of protein crystals and biodegradable release matrix

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Introduction

Poly-ε-caprolactone (PCL) is an excellent polymer for electrospinning as well as matrix-controlled drug delivery. It combines optimal processability using various techniques and good biocompatibility.

Electrospinning of many proteins has shown to be challenging because organic solvents are often required for efficient electrospinning of common polymers. However, such solvents may detrimentally affect protein integrity and/or stability. Furthermore, direct contact between encapsulated proteins and polymers may result in protein degradation. Formation of protein crystals is a potential solution due to higher thermodynamic stability and lower contact surface of crystals compared to amorphous protein.

We used lysozyme as readily crystallizing model protein. Suspensions of lysozyme crystals in PCL solution were electrospun resulting in different combinations of crystal size and fiber diameter. Furthermore, different amounts of poly(ethyleneglycol) (PEG) were admixed to adjust the porosity of the matrix. This model system was used to investigate lysozyme release profiles in dependence of fiber diameter, crystal size and porogen concentration.

Results and discussion

Lysozyme crystallization conditions were optimized such that stable crystal suspensions with monomodal size distributions, ranging from 700nm up to 70 µm, were obtained with high yield. The electrospinning process variables were optimized to generate PCL fibers of well defined diameters with narrow size distribution and high reproducibility.

We found that the burst effect could be well controlled by the fiber diameter and crystal size. In systems without porogen addition, release rates following burst release were constant and independent from crystal size or fibre diameter. By porogen addition protein release could be increased without significantly affecting burst release patterns.

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Surfactants for the formulation of NLC: Effects on size and physical stability

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Introduction
Nanostructured lipid carriers (NLC) are carrier systems for improved delivery of actives [1]. The efficacy is typically tested in cell culture tests. These media typically contain electrolytes, which can lead to a reduction of the zeta potential and thus to agglomeration of the particles [2]. The aim of this study was to develop/improve a NLC formulation which is physically stable in cell culture medium.

Materials and Methods
NLC were coated with various surfactants via dispersing them in either non-ionic or ionic surfactant solutions. NLC dispersed in water were used as control. Each sample obtained was then dispersed in cell culture medium and incubated for 1h. Particle size analysis and zeta potential measurements were performed prior to and after the incubation.

Results and Discussion
Uncoated NLC were not stable in cell culture medium, i.e. within 1h the size increased from 40nm to 72nm. When particles were coated with 2% TPGS, Tween, PLX 407 or PVP40T a stabilization effect was observed, i.e. no or little changes in size were observed. Less stabilization was observed for the particles coated with only 1% stabilizer. Coating the particles with Poloxamer 188 and SDS were not efficient for physical stabilization.

Zeta potential (ZP) analysis was in line with the results obtained from size analysis. Uncoated NLC analysed in water possessed a ZP of -30mV. Coating with non-ionic stabilizers led to a decrease in ZP, indicating the adsorption of the stabilizers onto the surface of the particles. The ZP further decreased with increasing surfactant concentration, i.e. thicker stabilizer layers were formed. The highest decreases in ZP were found for the most stabilizing surfactants TPGS, Tween, PLX 407 and PVP40T coated with 2% surfactant solution. Coating with SDS led to an increase in ZP to -70mV. However, upon addition of the cell culture medium it dropped down to -9mV, indicating insufficient stabilization of NLC by using ionic surfactants.

Conclusion
Coating NLC with non-ionic surfactant can improve their physical stability in cell culture medium. Zeta potential analysis is a suitable method to predict the stabilization efficacy of the surfactant.

References
Studying the influence of the charge and secondary structure of a cell-penetrating peptide over the uptake and cytotoxicity of drug-peptide conjugates

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Introduction
The cell membrane represents an impermeable barrier to many biologically active molecules and carrier systems. Two decades ago, a group of peptides collectively known as cell-penetrating peptides (CPP), have been characterized for their ability to translocate into living cells and to deliver different type of cargo into cells. The efficiency and mechanism of cell uptake of CPPs is strongly dependent on their charge as well as on the cargo. The attachment of small molecules like fluorophores or the addition of hydrophobic component can significantly influence not only the cellular uptake and distribution of CPPs, but also the overall toxicity. Therefore, one important aspect that should be considered when designing novel drug-peptide conjugates is the influence of the cargo over the interaction of any CPP with cells.

Results and discussion
In this study we investigated two doxorubicin-CPP conjugates that differ in their net charge at physiological pH, where one of them represents the first example of a conjugate between negatively charged CPP and a drug.

In addition, the conjugates were subject to molecular dynamics simulations in aqueous solution. Different molecular parameters were estimated so as to characterize the structures of the free compounds and how they change and influence one another after binding.

Cell-penetrating peptide drug conjugates
Finally, the uptake and cytotoxicity of the conjugates was evaluated against MCF-7 and HT-29 cell lines. The two different doxorubicin CPP conjugates possess very promising cytotoxic properties, even though the cellular uptake of the peptide drug conjugates was lower, determined by flow cytometry. Cytostatic effects and cellular uptake of the positively charged CPP doxorubicin conjugate appear superior compared to the negatively charged cell penetrating peptide conjugate.

References
Scalability of Nanosizing Using Wet Ball Milling

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Introduction

Miniaturization is widely used nowadays in the context of nanosuspension development, e.g. during stabilizer screening and milling feasibility studies. However, the usefulness of this miniaturized equipment to predict the result of larger scale equipment is still not very well established. In this research, we wanted to see whether it is possible to achieve a similar particle size reduction at different scales and with different mills. Therefore nanomilling was performed at gram-scale using a low energy wet ball milling (LE-WBM) and at larger scale with high energy wet ball milling (HE-WBM). Furthermore, it was also investigated whether the final particle size was affected by the milling principle, i.e. when the HE-WBM was performed in batch mode or recirculation mode.

Materials and Methods

Coarse suspension of 5% hesperetin (Exquim, S.A., Barcelona, Spain) in an aqueous 2% poloxamer 188 dispersion medium (Molekula, Dorset, UK) was nanosized in a miniaturized device (LE-WBM, suspension capacity of 15g) with a magnetic stirrer at 900 rpm for 5 hours.

HE-WBM was conducted in an agitated ball mill (Bühler PML2) either in batch mode (HE-BM) processing 120g suspension or in recirculation mode (HE-RM) processing 2kg suspension. The HE-WBM experiments were conducted at 1000 rpm and 2000 rpm. The milling time was up to 5h. Samples were withdrawn every 30 minutes and characterized by using photon correlation spectroscopy (PCS), laser diffractometry (LD) and light microscopy (Leitz Orthoplan, Germany). In all experiments, yttrium-stabilized zirconium beads with a diameter between 0.4-0.6mm were used as milling media.

Results and Discussion

Nanosizing using LE-WBM needed up to 72 hours to reach the minimal particle size. Similar results could be obtained for HE-BM at 1000 rpm after 2 hours. HE-BM milling at 2000 rpm resulted in even smaller particle sizes. HE-RM at 1000 rpm did not lead to similar particle sizes even after 5 hours of milling. When HE-RM was performed at 2000 rpm acceptable particle sizes could be reached. Overall it can be stated that HE-BM led to the best particle size results of about 120 nm. Scale up by using HE-WBM in recirculation mode will significantly increase the required milling times to reach a similar particle size reduction. To some extent the shorter residence time of the particles in the mill can be compensated by higher energy input, e.g. via increasing the rpm of the agitator. In general, all 3 methods resulted in nanosuspensions with small particle sizes.

References

B.V Eerdenburgh, et.al ; Int.J.Pharm., 364 (2008) 64-75
Properties of biodegradable polymer films and controlled drug release

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Introduction
An often emerging consequence of abdominal and pelvic surgery is the formation of adhesions, which are abnormal fibrous connections between two anatomically different and usually separated surfaces. Such adhesions can result in severe problems like abdominal and pelvic pain, bowel obstructions and infertility [1]. Therefore, efforts have been made to prevent those adhesions with pharmacotherapy or physical barrier devices. The aim of this study was to improve the mechanical properties and degradation of the polylactide [PLA] films as well as to load the films with antibiotic drugs in order to combine the abovementioned anti-adhesive approaches.

Methods
PEG-PLA copolymers with varying PLA chain lengths and poly(ethylene glycol) [PEG] geometry were synthesized via standard ring-opening polymerization and characterized via GPC, ¹H-NMR and DSC. The Polymer films were solvent cast on sigmacote-coated glass plates and dried in vacuo before use. To determine the films’ mechanical properties, tensile and puncture tests were performed on a texture analyser. Degradation studies were conducted over 8 weeks at 37°C. Drug release was investigated with RP-HPLC analysis and additional microbiological testing of drug loaded films on bacteria containing agar.

Results and Discussion
The copolymerization of PLA with PEG led to softer films with shorter degradation times due to the plasticizing effect of PEG and its hydrophilicity. The increased water uptake also facilitated the initial drug release, but did not affect the long term release (Fig.1).

Conclusions
Copolymerization of PLA with PEG allows adjusting the mechanical and degradation properties of anti-adhesive films. In order to control the long term drug release from these films further polymer modifications might become necessary.

Acknowledgements
Thanks to MAST Biosurgery AG for financial support and to Prof. A. Goepferich for the possibility to conduct parts of this work in his lab.

References
Controlled drug delivery from thin biodegradable polymer films

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Introduction
Systemic treatments of strictly localized surgical wounds often come along with undesired side effects [1]. Therefore a local application of active agents is highly desired in such cases. The aim of this work was to investigate a film delivery device for a local application after peritoneal surgery that can be applied directly to the trauma site. Depending on the applied drugs, a faster or a slower release may be desirable, which was studied by using different types of polymers as vehicles.

Materials and Methods
Different hydrophilic or lipophilic polymers were used to control the release of vancomycin-hydrochloride. The hydrophilic drug was incorporated in the lipophilic polymers by suspending the drug or by addition of a suitable cosolvens. Films were investigated using light and scanning electron microscopy (Figure 1). The release was studied in glass vials with small film discs in 10 ml HEPES incubated at 37°C. At predetermined time intervals, 1 ml buffer was removed and investigated for the released drug content.

Results and Discussion
Based on the chosen film preparation the drug distribution and crystal formation in the films could be varied. Accordingly, the release of vancomycin-hydrochloride exhibited a large burst from the hydrophilic alginate film, despite the possible ionic interactions. The lipophilic PLA on the other hand only exhibited a small initial drug release, due to the small amounts of water which could permeate into the lipophilic film to dissolve the drug. The alternative PLA-PEG-PLA triblock with included hydrophilic parts showed a slightly larger initial burst, but still leveled off after the first day of release. Based on the chosen preparation method (suspension or cosolvens) the distribution of the drug as well as its release could be further modified to adjust release kinetics.

Conclusion
The choice of degradable polymers as well as the preparation method can serve as a versatile tool to influence the drug release kinetics from thin polymer films.

Acknowledgements
Thanks for financial support by MAST Biosurgery AG and to Prof. A. Goepferich for the possibility to conduct parts of this work in his lab.

References
Spray encapsulation of PLGA nanoparticles for oral vaccination

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Introduction

Oral delivery is the preferred route of administration of drugs. However, vaccines administered by this route face several challenges. Carrier systems are needed that protect the antigen against proteolysis in the gastrointestinal tract, foster mucosal transport and enable uptake into antigen-presenting cells to elicit a strong systemic immune response. In this study we investigate the feasibility of gastroresistantly coated PLGA nanoparticles as delivery vehicle for oral vaccination.

Experimental Methods

PLGA nanoparticles were manufactured by the double emulsion solvent evaporation method. Ovalbumin was incorporated as model antigen. Particles were washed by centrifugation and resuspended in Milli-Q grade water. Mean particle diameters were determined by dynamic light scattering (Malvern Zetasizer) and laser diffraction (Horiba LA-950). Protein content was determined by BCA assay.

The nanoparticle suspensions were encapsulated into microparticles by spray drying (ProCepT 4M8-TriX) using different excipients to increase the yield and the redispersibility. The dried powder was further characterized regarding particle size, size distribution and residual water content. After dispersion in ethanol containing Eudragit\textsuperscript{®} L, the particles were spray-dried a second time to form a gastroresistant multiparticulate delivery system.

Results and Discussion

Mean diameters of the nanoparticles were around 200 nm with polydispersity indices below 0.2. Spray-dried particles were spherical and evenly shaped with diameters between 1-5 µm. Powder formulations containing trehalose as bulking agent and leucine as dispersing aid [1] were fully redispersible without affecting mean particle sizes.

Further spray encapsulation of PLGA/trehalose microparticles into Eudragit\textsuperscript{®} yielded rugged, unevenly shaped particles with diameters between 5-20 µm. Relatively high amounts of Eudragit\textsuperscript{®} (4 times the weight of encapsulated microparticles) were necessary to effectively prevent nanoparticle release in 0.1N HCl.

Further optimization is ongoing to reduce the amount of coating. Larger composite particles may be favorable for the coating/core ratio and could be achieved by using nozzles with bigger orifices. An alternative approach to directly encapsulate the PLGA nanoparticles into Eudragit\textsuperscript{®} microparticles will be investigated and also release, distribution and immunogenicity systematically studied.

Acknowledgements

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References

Novel Techniques for Shedding Light upon the Interaction between IgG and HPβCD

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Introduction

Hydroxypropyl-β-cyclodextrin (HPβCD) offers a great capacity to stabilize IgG against surface-induced damage. [1] Meanwhile, a surfactant-like behavior similar to polysorbate 80 was refuted recently. [2] In this study, two novel techniques - namely quartz crystal microbalance (QCM) and static/dynamic light scattering - were applied to investigate the hypothesis of a direct interaction between IgG and HPβCD.

Materials and Methods

QCM-experiments were performed on a QCM200-device (Stanford Research Systems) by using 5 MHz gold crystals. A sample volume of 250 µL was injected onto the crystal, and protein adsorption was monitored until the equilibrium was achieved.

Static/dynamic light scattering measurements were performed by Zetasizer Nano-ZS (Malvern Instruments). For each HPβCD-concentration, a protein-concentration series of 10, 8, 6, 4, and 2 mg/mL was measured for calculating the protein-interaction parameters B_{22} and k_o.

Results and Discussion

a) Quartz crystal microbalance

Presence of HPβCD leads to a reduced IgG-adsorption to the gold crystal (Figure 1). Since protein adsorption to gold is mainly driven by hydrophobic amino acid residues, decreased protein adsorption in presence of HPβCD indicates a direct interaction between the cyclodextrin and IgG which leads to a shielding of hydrophobic sites on the protein surface.

b) Static/dynamic light scattering

Increasing B_{22}-values show that addition of HPβCD up to 100 mM leads to a change in intermolecular protein forces from attractive to repulsive (Figure 2). This can be seen as further confirmation for an HPβCD-IgG-interaction.

Conclusions

Measuring the interaction between IgG and HPβCD was successful by using QCM and light scattering techniques. Both methods indicate a shielding of hydrophobic parts on the protein surface and therefore a direct interaction between HPβCD and the IgG.

References

Methylaminobutyrate substituted glucose based non-viral vectors for gene delivery

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Abstract

Natural polymers for gene delivery favor the advantage of good biocompatibility and their renewable production source. In this study cationization of dextran and cellulose was achieved by an innovative one step reaction at room temperature by esterification of the N-methyl-2-pyrrolidone and p-toluensulfonyl chloride yielding methylammonium group as substituent.[1] The polymers were characterized regarding their cyto- and hemo-compatibility. [2] Complexes were prepared using luciferase reporter gene (plasmid pGL3) in varying polymer nitrogen to DNA phosphate (N/P) ratios. Physicochemical complex characteristics were investigated measuring zeta potential and hydrodynamic diameter, DNA binding capacity and protection against enzymatic degradation. Additionally transfection efficiency was tested. Cationized dextrans proofed to be biocompatible at all tested degree of substitution (DS) and concentrations compared to cationized cellulose, which showed cyto- as well as hemo toxicity at DS of 0.98 and concentrations of 500 µg/mL. All polymers demonstrated DNA binding capacity even after freeze-drying and freezing the complexes at -80 °C, but insufficient protection of plasmids against DNase. Size and zeta potential could be measured for dextran/DNA complexes at about 100 nm and 15 to 50 mV. Cellulose/DNA complexes were characterized by a high PDI value. Transfection of pGL3 increased with increasing DS with the ranking dextran/DNA >0.52. Obtained results proofed suitability of methylaminobutyrate substituted polysaccharides for the use as non-viral vectors for gene delivery, depending on DS, N/P ratio and the used polymer backbone, with dextran revealing biocompatibility and higher transfection efficiency compared to cellulose.

References


Acknowledgments

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“Nano Q10” as Commercial intermediate product for Cosmetics

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Introduction

Since a few years coenzyme Q10 loaded onto nanostructured lipid carriers (NLC) is a product in cosmetics on the market. The dermal penetration capacity of Nano Q10 is better than Q10-loaded nanoemulsion or as Q10 solution (e.g. in liquid paraffin) [1]. As intermediate product, pre-manufactured NLC concentrates are convenient to the cosmetic companies. However, the concentrates need to be preserved, and the physical stability of Q10-loaded NLC can be impaired by antimicrobial agents. To identify the most suitable preservatives, a long term physical stability study was performed.

Materials and Methods

Hot high pressure homogenization was used to produce Q10-loaded NLC suspensions at the conditions of 85 °C and 800 bar (4.8% Q10, 14.45% Cutina CP, 0.75% Miglyol 812, with 1.8% TegoCare 450). Low irritative preserving agent or their mixtures (9 formulations) were admixed to NLC concentrates. To monitor physical stability, light microscopy, photon correlation spectroscopy (PCS, Zetasizer Nano ZS, Malvern, UK) and laser diffraction (LD, Mastersizer 2000, Malvern, UK) have been performed. Polymorphism modifications were determined by Differential Scanning Calorimeter (DSC) (DSC821e, Mettler Toledo, Germany).

Results and discussion

Except the one with caprylyl glycol, all nano Q10 NLC concentrates showed similar diameter (about 210nm) but slightly increased PI after 6 months. LD measurements confirmed their stability by LD50% 0.210µm and LD99% 0.650µm. No large particles or aggregates > 1 µm could be observed by light microscopy. DSC heating results showed the same melting peaks as on day 1, indicating also stability of the particle matrix. Nano Q10 admixed of caprylyl glycol was aggregated immediately and DSC results showed splitting of peaks in the heating curves after 6 months.

Conclusion

Low irritative preserving agents like pentylene glycol etc. provide antimicrobial efficiency, and are in the preservative-free trend in the cosmetic industry. With destabilizing preserving agents polymorphic transitions of lipids were observed, which are initiators or accompanying effects of instabilities in NLC suspensions, as reported previously [2].

References

Effect of manufacturing process on particle size of Cilengitide suspensions

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Introduction
A microparticle-based, parenteral suspension of Cilengitide, an integrin receptor inhibitor, is in development for an advanced clinical use (self-application) [1]. In this study, particle size of suspensions with micronized and non-micronized API was determined to investigate the influence of preparation and storage as well as to reassess a micronization step.

Materials and Methods
The formulations contained 2 mg/ml DMPG (Dimyristoylphosphatidylglycerol-Sodium, Lipoid D-Ludwigshafen) and 60 mg/ml of micronized and non-micronized API (Merck, D- Darmstadt). The micronized suspensions were additionally treated with ultrasound (Sonopuls HD 2070, Bandelin, D-Berlin). The particle size distributions were determined by laser diffraction with a Mastersizer 2000 (Malvern, D-Herrenberg).

Results
The size distribution of micronized API was unaffected by the duration of stirring. In contrast, stirring reduced the particle size of non-micronized API (Fig. 1).

Fig. 1: Influence of stirring (non-micronized API)

Longer stirring (48 h) of formulations with non-micronized API led to size distributions comparable to suspensions with micronized API, whereby particles partly dissolved (saturation level reached after 5 min) and broke into smaller parts at predetermined break points shown by stereomicroscopy (Fig. 2).

Fig. 2: Disintegration of a non-micronized crystal

Application of ultrasound did not result in further reduction of the particle size of micronized API.

Fig. 3: Particle size of treated / untreated suspension

Suspensions with non-micronized and micronized API showed an acceptable particle size stability for a period of one year.

Conclusion
Stirring of suspensions containing non-micronized API resulted in a comparable particle size distribution to micronized API. However, stirring times up to 48 h were necessary. All preparations were acceptably stable during storage. Based on these results it can be concluded that a micronization step has no advanced effect on the particle size, but shortens the manufacturing process.

References
Lipid nanoparticles formulations of virgin coconut oil for atopic dermatitis treatment

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Introduction
Atopic dermatitis (AD) is a skin disease which shows dry skin due to distorted barrier function. Colonization of *Staphylococcus aureus* was also reported in patients with AD. A recent clinical trial confirmed that regular application of virgin coconut oil can treat the symptom of atopic dermatitis and reduce the *S. aureus* colonization [1]. Nanostructured lipid carriers (NLC) are a carrier system which can carry active oil. The very small size and solid nature at body temperature of lipid nanoparticles provide high skin occlusion to repair the imperfect skin barrier without oily feeling on the skin [2]. To improve the treatment of atopic dermatitis, we formulated virgin coconut oil (VCO) into lipid nanoparticles. PEG-free surfactants are used in this study because PEG-containing surfactant should not be applied on damaged skin which is the characteristic of AD [3].

Materials and Methods
Cutina® CP and Dynasan® 118 were used as the solid lipid. Three types of Plantacare® were used as the surfactant. The melted solid lipid (10%) and VCO (10%, total particle concentration in dispersion 20%) was mixed with surfactant solution to produce a pre-emulsion, then it was passed through a high pressure homogenizer Micron LAB 40 (500 bar, 3 cycles, 85°C). The formulations were cooled at room temperature. The particle size and polydispersity index were determined using photon correlation spectroscopy. Differential scanning calorimetry (DSC) was used to characterize the melting point of the formulations. The formulations were kept at room temperature and 4°C for three months of the physical stability study.

Results and Discussion
The particle size and polydispersity index decreased as the number of cycles increased. Nanoparticles were produced with particle size of all formulations in the range of 160-220 nm. A narrow particle size distribution was obtained from all formulations (polydispersity index <0.2). The particle size of formulations using Cutina® CP, Plantacare® 2000 UP and Plantacare® 1200 UP did not increase during three months storage in room temperature. A melting peak was not observed or very weak for lipid nanoparticles formulations using Cutina® CP when kept at room temperature. However, a melting peak was observed from the formulations kept in the refrigerator. The NLC were re-crystallized. This shows that cooling process/storage temperature can be critical parameters to produce lipid nanoparticles with high content of oil.

Conclusions
NLC formulations of virgin coconut oil were successfully developed in this study. The particle size is around 200 nm and they are physically stable for 3 months.

References
Nanostructures of monocationic peptides as a result of self-aggregation and complexation

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**Introduction**

We recently reported that monocationic peptides like Ozarelix form rodshaped nanocomplexes with negatively charged linear biopolymers. These chainlike nanorods consist of polymers such as alginate or nucleic acids and are coated with peptide molecules [1]. Here, as an alternative to Ozarelix we choose Cetrorelix. The goal was to investigate the influence of modifications of the peptide component on size and shape of the nanostructures as well as thermodynamic properties of the complex formation.

**Materials and Methods**

Complex formation is initiated by simple mixing the two dissolved components. We analyze the structure of peptidic self-aggregates and peptide-polymer-complexes by negative staining Transmission electron spectroscopy (TEM) and cryo-TEM imaging. Hydrodynamic diameters of dispersed complexes at different charge ratios are measured using Dynamic light scattering. Isothermal titration calorimetry is used to determine the thermodynamic properties of the complex formation process.

**Results and Discussion**

We show that, in contrast to Ozarelix, the complexation of Cetrorelix and anionic biopolymers follows a different mechanism. Cetrorelix leads to more stable self-aggregates, which are then used as a template for the biopolymer binding. Cryo-TEM images nicely illustrate these nanostructures, which consist of very long twisted nanofibers with 8 nm in diameter and over 300 nm in length.

**Conclusion**

We show that Cetrorelix forms stable self-aggregates, which are used as a template during complex formation with oppositely charged polymers.

In a next step, the molecular build-up of the complexes will be clarified by molecular modeling calculations.

**References**

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[Section] Introduction

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References
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